Acute adaption to oral or intravenous phosphate requires parathyroid hormone

Thomas, Linto ; Bettoni, Carla ; Knöpfel, Thomas ; Hernando, Nati ; Biber, Jürg ; Wagner, Carsten A

Abstract: Phosphate (Pi) homeostasis is regulated by renal, intestinal, and endocrine mechanisms through which Pi intake stimulates parathyroid hormone (PTH) and fibroblast growth factor-23 secretion, increasing phosphaturia. Mechanisms underlying the early adaptive phase and the role of the intestine, however, remain ill defined. We investigated mineral, endocrine, and renal responses during the first 4 hours after intravenous and intragastric Pi loading in rats. Intravenous Pi loading (0.5 mmol) caused a transient rise in plasma Pi levels and creatinine clearance and an increase in phosphaturia within 10 minutes. Plasma calcium levels fell and PTH levels increased within 10 minutes and remained low or high, respectively. Fibroblast growth factor-23, 1,25-(OH)2-vitamin D3, and insulin concentrations did not respond, but plasma dopamine levels increased by 4 hours. In comparison, gastric Pi loading elicited similar but delayed phosphaturia and endocrine responses but did not affect plasma mineral levels. Either intravenous or gastric loading led to decreased expression and activity of renal Pi transporters after 4 hours. In parathyroidectomized rats, however, only intravenous Pi loading caused phosphaturia, which was blunted and transient compared with that in intact rats. Intravenous but not gastric Pi loading in parathyroidectomized rats also led to higher creatinine clearance and lower plasma calcium levels but did not reduce the expression or activity of Pi transporters. This evidence suggests that an intravenous or intestinal Pi bolus causes rapid phosphaturia through mechanisms requiring PTH and downregulation of renal Pi transporters but does not support a role of the intestine in stimulating renal clearance of Pi.

DOI: https://doi.org/10.1681/ASN.2016010082

Parathyroid hormone is critical for the acute adaption to oral or intravenous phosphate loading

Linto Thomas, Carla Bettoni, Thomas Knöpfel, Nati Hernando, Jürg Biber, and Carsten A. Wagner

Institute of Physiology, University of Zurich, Switzerland and National Centre for Competence in Research NCCR Kidney.CH, Switzerland

Corresponding author
Carsten A. Wagner
Institute of Physiology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
Switzerland
Phone: +41-44-63 55023
Fax: +41-44-63 56814
E-mail: Wagnerca@access.uzh.ch

ABSTRACT

Phosphate (Pi) homeostasis is regulated by renal, intestinal, and endocrine mechanisms where Pi intake stimulates PTH, FGF23, and phosphaturia. The mechanisms underlying the early adaptive phase and the role of the intestine remained ill defined. We investigated the mineral, endocrine, and renal response to intravenous and intragastric Pi loading in rats during the first 4 hours after administration. Intravenous Pi at the highest dose (0.5 mmol) caused a transient rise in plasma Pi, phosphaturia within 10 minutes accompanied by a transient increase in creatinine clearance. Plasma calcium fell and PTH increased within 10 minutes remaining low or high, respectively. FGF23, 1,25-$(OH)_2$-vitamin D_3, and insulin did not respond but dopamine increased by 4 hours. Gastric Pi-loading had no effect on plasma Pi and calcium levels but elicited a similar phosphaturia and endocrine responses as intravenous application with a delay of 20-30 minutes. Intravenous or gastric loading was paralleled by decreased expression and activity of renal Pi transporters after 4 hours. NaCl loading had no effect on all parameters. The role of PTH was examined in parathyroidectomized (PTX) rats where only intravenous Pi loading caused a blunted and transient phosphaturia paralleled by higher creatinine clearance and a more pronounced fall in plasma calcium. Pi-loading did not reduce expression and activity of Pi transporters in PTX rats. Thus, an intravenous or intestinal Pi bolus causes rapid phosphaturia depending on elevated PTH and downregulation of renal Pi transporters. There is no evidence supporting a role of the intestine in stimulating renal clearance of Pi.

INTRODUCTION

Phosphate (Pi) homeostasis is achieved by the balanced actions of intestinal absorption, renal reabsorption, and bone mineralization/demineralization. Intestinal absorption and renal reabsorption of Pi are mostly mediated by Na^+-dependent Pi (Na/Pi) cotransporters from the SLC34 family of solute carriers, consisting of the renal NaPi-IIa and NaPi-IIc, and the intestinal NaPi-IIb transporters[1]. The activity and expression of these transporters is downregulated in response to increased dietary Pi intake whereas Pi deficiency promotes their upregulation[1-2]. The adaptive regulation of renal and intestinal Pi transport is at least in part mediated by several hormones including fibroblast growth factor-23 (FGF23), 1,25-dihydroxy-vitamin D_3 (1,25-$(OH)_2$-vitamin D_3), parathyroid hormone (PTH), insulin and dopamine[3-7]. PTH and FGF23 rise in response to Pi intake and alone or together downregulate renal Pi transporters increasing phosphaturia[6, 8]. Similarly, dopamine stimulates renal Pi excretion by reducing renal Pi transporter activity and expression [9-11]. 1,25-$(OH)_2$-vitamin D_3 levels increase during Pi deficiency and enhance renal and intestinal Pi absorption [8, 12-14]. Also insulin stimulates expression of renal Pi transporters and reduces phosphaturia[15]. The actions of FGF23, PTH, and 1,25-$(OH)_2$-vitamin D_3 are coupled through multiple negative and positive feedback loops [6, 8, 16].

However, the exact roles of these hormones in the acute and chronic response to changes in Pi intake are not fully defined. Moreover, the existence and role of additional factors and mediators in the control of Pi homeostasis have been proposed[17-18]. Klotho, a cofactor for FGF23 signaling, also exerts direct effects on renal Pi and calcium transporters independent from FGF23[6, 19-20]. MEPE and sFRP4 may act on renal and extrarenal targets to lower plasma Pi[21-23]. Also the existence of a putative intestinal phosphaturic factor has been postulated based on the rapid phosphaturic effect of duodenal Pi infusion, the absence of changes in plasma Pi, PTH and FGF23, its independence from renal innervation, and the phosphaturic effect of duodenal extracts from Pi infused rats [24]. However in humans, acute enteral and parenteral Pi loads cause

dose dependent changes in phosphaturia only upon changes in plasma Pi and PTH and a similar phosphaturic response is observed with both routes of Pi administration. Thus, in contrast to rodents, the human data is not consistent with the presence of an intestinal Pi sensor [25]. Local Pi sensing mechanisms in kidney and other organs may be involved in sensing changes in dietary Pi intake and may mediate or modulate some of the effects on renal Pi handling. Evidence for Pi sensing has been obtained from isolated parathyroid gland cells in vitro [26-27], the opossum kidney derived OK cell line [28-29], bone and vascular cells [30-32]

The aim of this study was to investigate the endocrine, mineral, and renal responses to acute Pi loading in rats and to test for evidence for a role of the intestine in determining the adaptive response by administering Pi either intravenously or intragastrically.

RESULTS

Phosphate loading rapidly elicits phosphaturia

Intravenous infusion of a Pi bolus (500 μmoles Pi) caused a rapid and transient increase in plasma Pi concentration in intact rats (Figure 1A) with a peak after 10 minutes. Two hours after infusion, plasma Pi had returned to baseline values. A significant, although blunted increase in plasma Pi was observed 10 minutes upon infusion of 150 μmoles but not with 50 μmoles Pi (supplementary Figure 1A). In contrast, plasma Pi did not change in rats gavaged with 500 μmoles Pi (Figure 1A). The concentration of plasma Pi remained normal after intravenous or intragastric administration of 500 or 150 μmoles NaCl (Figure 1A and supplementary Figure 2A).

Intravenous infusion of 500 μmoles Pi rapidly and strongly increased urinary Pi excretion within 10 minutes. Although phosphaturia tended to decrease after 30 minutes, it remained high over 4 hours after administration (Figure 1B). Infusions of 150 μmoles and 50 μmoles Pi failed to induce any significant phosphaturic response (supplementary Figure 1B). Intragastric administration of 500 μmoles Pi increased urinary excretion of Pi to a similar extent as intravenous administration, however, the onset of phosphaturia was delayed compared to infusion, reaching significance only 60 minutes after the Pi bolus (Figure 1B). Phosphaturia remained high over 4 hours post gavage (Figure 1B). Changes in the fractional excretion of Pi after the Pi bolus paralleled urinary Pi concentration (supplementary Figure 3A). Neither intravenous infusion nor gavage of 500 or 150 μmoles NaCl affected the urinary excretion of Pi (Figure 1B and supplementary Figure 2B).

Creatinine clearance significantly increased within 10 minutes after intravenous application of 500 μmoles Pi and thereafter rapidly normalized (Figure 1C). Intragastric loading with 500 μmoles Pi did not alter creatinine clearance (Figure 1C). Similarly, administration of 500 or 150 μmoles NaCl by infusion or gavage had no effect (Figure 1C and data not shown).

The ~~ac~~cumulative urinary excretion of Pi showed a similar level after 4 hours of the oral or intravenous bolus (Figure 1D). Although a delay was observed in the gavage group, both groups had excreted comparable amounts of Pi four 4 post application, with mean values of around 230 and 280 μmoles Pi, respectively, representing about 50-60% of the initial Pi load (500 μmoles).

In order to examine possible organ storage of the non-excreted Pi, we assessed the Pi content in femurs, liver and skeletal muscle in tissues from rats infused and gavaged with Pi or NaCl. As expected, the concentration of Pi in femurs was higher (10 to 20 times) than in the other two organs (Supplementary Figure 4). However, in all three tissues, similar levels of Pi were measured in samples from control and Pi-loaded rats.

The concentration of total Ca^{2+} in plasma slightly but significantly decreased upon intravenous infusion of 500 μmoles Pi (Figure 2A). The reduction was already detectable 10 minutes after the Pi bolus and although it tended to normalize at the latest time points, plasma Ca^{2+} remained low until the end of the experiment (4 hours). Similar changes were observed upon Pi infusion with 150 μmoles Pi, but not with 50 μmoles Pi (supplementary Figure 1C). In contrast, total plasma Ca^{2+} did not change significantly after intragastric administration of 500 μmoles Pi (Figure 2A). Infusion or gavage with either 500 or 150 μmoles NaCl did not alter total plasma Ca^{2+} (Figure 2A and supplementary Figure 2C).

The urinary excretion of Ca^{2+} remained unchanged after intravenous or intragastric Pi or NaCl application (Figure 2B, supplementary Figures 1D and 2D). However, infusion with Pi or NaCl caused a small, non-significant, and transient increase in urinary Ca^{2+} excretion possibly reflecting an acute volume load (Figure 2B).

Phosphate loading causes an immediate PTH rise

In intact animals, intravenous infusion of 500 μmoles Pi increased plasma intact PTH levels six-fold within 10 minutes, whereas in gavaged rats a two-fold increase was detected after 25 minutes paralleling or even preceding the increase in urinary Pi

excretion (Figure 3A). In both cases, PTH remained high 4 hours after Pi-administration (Figure 3A). No changes in the plasma levels of intact FGF23 (Figure 3B), 1,25-$(OH)_2$ vitamin D_3 (Figure 3C) and insulin (Figure 3D) were observed in rats after Pi administration. Plasma dopamine significantly increased at 4 hours after 500 μmoles Pi infusion but not gavage (Figure 3E) whereas urine dopamine was not altered (Figure 3F). Infusion and gavage with saline had no effect on the hormones analyzed.

The phosphate load reduces renal phosphate transporter activity and expression

Flux measurements of ^{32}Pi and ^{3}H-D-glucose were performed in BBMV isolated from kidneys collected 40 minutes and 4 hours after 500 μmoles Pi or saline application to assess proximal tubular luminal phosphate transporter activity. In BBMV from kidneys extracted 40 minutes after application, all ^{32}Pi uptake components were similar in Pi loaded and control samples (Figure 4A and B). Similarly, no differences were detected for the Na^+-dependent uptake of D-glucose (Figure 4E). In contrast, the total Na^+-dependent as well as SLC34-mediated ^{32}Pi-transport activities were reduced in kidneys 4 hours after infusion with 500 μmoles Pi (Figure 4C). ^{32}Pi fluxes also decreased, though non-significantly, in the Pi-gavaged animals after 4 hours (Figure 4D). The Na^+-dependent uptake of D-glucose remained unaffected 4 hours in Pi infused or gavaged animals (Figure 4F).

The abundance of the major renal Pi transporters NaPi-IIa and NaPI-IIc in renal BBMV isolated 40 minutes after infusion or gavage with Pi or saline was unchanged (Figure 5A and 5B). The abundance of both cotransporters was significantly reduced in kidneys collected 4 hours after infusion with 500 μmoles Pi (Figure 5C), whereas the expression of NaPi-IIa but not NaPi-IIc was diminished 4 hours after intragastric loading (Figure 5D).

Parathyroid hormone is required to clear the acute phosphate load

Since PTH increased very rapidly upon Pi loading and paralleled or even preceded phosphaturia, we tested the role of PTH in the adaptive response in parathyroidectomized (PTX) rats. Infusion of 500 μmoles Pi, caused a rapid increase in

plasma Pi in PTX rats (Figure 6A) peaking after 10 minutes and decreasing thereafter. However, whereas the levels of Pi fully normalized within 2 hours post-infusion in intact rats, Pi levels remained elevated until the end of the experiment in PTX animals (Figure 6A). Gavage of 500 μmoles Pi in PTX rats induced a slow rise in plasma Pi (Figure 6A) in contrast with the lack of effect of the Pi gavage in intact animals (Figure 1A). Administration of saline to PTX rats by infusion or gavage did not alter plasma Pi (Figure 6A).

Infusion with 500 μmoles Pi induced a fast but small increase in urinary excretion of Pi in PTX rats (Figure 6B). Maximal phosphaturia was detected 10 min post-infusion similar to intact animals (Figure 1B). However, phosphaturia returned to baseline within 2 hours after infusion in PTX rats despite elevated plasma Pi levels (Figure 6B). Moreover, gavage of Pi in PTX rats failed to elicit any significant phosphaturia (Figure 6B). Neither infusion nor gavage with saline affected urinary Pi (Figure 6B).

In PTX rats, creatinine clearance increased within 10 minutes of 500 μmoles Pi infusion and rapidly normalized thereafter, whereas it was not altered with either Pi gavage or saline administration (Figure 6C).

The ~~ac~~cumulative urinary excretion of Pi over 4 hours in PTX rats infused with 500 μmoles Pi was of about 30 μmol (Figure 6D) equivalent to about 6% of the Pi load. The ~~ac~~cumulative urinary excretion of Pi over 4 hours in PTX rats receiving Pi by gavage was about 3 μmol (Figure 6D), representing less than 1% of the Pi load.

The content of Pi was higher in femurs than liver and muscle in PTX animals, and no differences were found between organs extracted from saline treated and Pi-loaded rats (Supplementary Figure 5).

Plasma total Ca^{2+} decreased slightly within 10 minutes upon 500 μmoles Pi infusion in PTX rats (Figure 7A) and remained low until the end of the experiment. In contrast, plasma total Ca^{2+} did not change significantly in Pi gavaged PTX rats (Figure 7A). Administration of 150 μmoles NaCl by infusion or gavage did not alter plasma Ca^{2+}.

Urinary excretion of Ca^{2+} in PTX showed no significant changes after administration of Pi or saline (Figure 7B).

As expected, plasma PTH was undetectable in PTX rats under all conditions (Figure 8A). In contrast to intact rats, FGF23 was elevated in PTX animals 4 hours after oral or intravenous Pi loading (Figure 8B). The plasma concentrations of 1,25-$(OH)_2$-vitamin D_3 (Figure 8C), insulin (Figure 8D) and dopamine (Figure 8E) as well as of urine dopamine (Figure 8F) remained unchanged under all conditions.

Parathyroid hormone downregulates renal phosphate transporters in response to acute phosphate loading

Flux measurements of ^{32}Pi and ^{3}H-D-glucose in BBMV from PTX rat kidneys collected 4 hours post-loading were similar in samples from Pi-loaded and controls (Figure 9). The protein abundance of NaPi-IIa and NaPi-IIc in PTX rats were similar in the Pi loaded and saline treated animals, 4 hours post-administration (Figure10).

DISCUSSION

Acute and chronic changes in plasma Pi elicit adaptive responses in several hormones that regulate renal and intestinal epithelia (re)absorbing Pi [1-2, 8]. High dietary Pi increases the phosphaturic hormones PTH, FGF-23, and dopamine while decreasing the levels of 1,25-$(OH)_2$-vitamin D_3 [7-9, 33] resulting in reduced expression and activity of Na/Pi cotransporters in renal and intestinal epithelia, blunting intestinal Pi absorption and increasing urinary excretion. However, there are conflicting data regarding the sequence of events triggered by high dietary Pi, as well as regarding the nature of the trigger itself. PTH may have a key role in the acute renal response, with other hormones coming into play only later on [25, 34-36], but the presence of yet-unidentified intestinal factor(s) stimulating renal Pi excretion independent from PTH has been proposed [37]. Here, we administered a Pi load to rats, both intravenously (to bypass the gastrointestinal tract) and intragastrically, and compared the acute responses in intact and PTX animals.

Our data demonstrate that in comparison to equimolar NaCl infusions and the time point zero the acute infusion of Pi elicited a dose-dependent response that consisted at the highest dose of 1) an immediate but transient rise in plasma Pi, 2) an early and transient increase in creatinine clearance, 3) a rapid phosphaturic response, 4) a fall in plasma total calcium, 5) a rapid and sustained phosphaturia, and 6) a reduced expression and activity of renal Pi transporters. Intragastric application of Pi caused a qualitatively similar response in the cumulative Pi excretion without a significant change in creatinine clearance, no obvious hyperphosphatemia, a lower and delayed rise in PTH, a non-significant reduction in renal Pi transporter activity and lower expression of only NaPi-IIa but not NaPi-IIc, and a slower onset in phosphaturia. Infusion or gavage of NaCl had no effects.

Several points are of major interest: the onset of phosphaturia is paralleled by a rise in PTH in infused and gavaged animals and precedes changes in plasma dopamine whereas levels of 1,25-$(OH)_2$-vitamin D_3, FGF23, insulin, and urine dopamine were not altered suggesting an important role of PTH. Early phosphaturia (40 minutes) occurred

in infused animals without obvious changes in the activity or abundance of renal Pi transporters expressed in the brush border membrane of the proximal tubule which may be explained at least in part by a higher tubular load in the infused animals as creatinine clearance had more than doubled and plasma Pi levels were elevated. However, we noted also a partial dissociation between the degree of phosphaturia, Pi-transport activities in brush border membrane vesicles, and the abundance of the NaPi-IIa and NaPi-IIc transporters as most evident in the Pi gavaged group after 4 hours. Activity of NaPi-IIa transporters in the brush border membrane is influenced by lipid composition of the plasma membrane, in-situ cleavage by klotho, and possibly by regulated association with and dissociation from NHERF1 through phosphorylation of NHERF1 stimulated by PTH or dopamine [19, 38-40]. We did not obtain evidence for cleavage of NaPi-IIa as evident from immunoblotting but changes in lipid composition or NHERF1 phosphorylation were not tested and may contribute to the dissociation of transport activity and NaPi-IIa abundance.

Plasma PTH rises *in vivo* and *in vitro* in response to a fall in ionized calcium or an increase in Pi concentrations. Whether the response to Pi is independent from calcium has remained unclear as Pi retains its ability to stimulate PTH secretion even in the absence of a measurable fall in ionized or total calcium[25-27, 35, 41]. Along the same line, in vitro incubation of human parathyroid glands with escalating concentrations of phosphate while keeping ionized calcium constant stimulated PTH secretion[26, 41]. On the other hand, inhibitors of Calcium-stimulated receptor (CaSR) mediated PTH secretion (calcimimetics) have been shown to suppress Pi induced PTH secretion in vivo suggesting a role of the CaSR even in the absence of changes of ionized calcium levels [27]. Here, plasma total calcium decreased in parallel with the rise in PTH allowing no clear distinction between a calcium dependent or independent mechanism. However, infusion of 150 μmoles Pi caused a similar fall in plasma total calcium as 500 μmoles but the rise in PTH was blunted and detected only 10 minutes post administration, suggesting that Pi may stimulate PTH release synergistically or independently. The early response of PTH found in our animal model is consistent with experiments in humans and other rodent models [25, 34-35].

PTH is critical for the early response to Pi, independent from the route of application. In PTX rats, massive and sustained hyperphosphatemia developed with a more pronounced fall in plasma total calcium than in intact rats. There was a small and transient increase in renal Pi excretion in the Pi infused PTX group that is most likely attributable to the combination of hyperphosphatemia and elevated glomerular filtration rate (as indicated by higher creatinine clearance). Phosphaturia ceased after normalization of creatinine clearance and the fall of plasma Pi below about 5 mM. The transient increase in creatinine clearance in the Pi infused animals is independent from PTH and may involve other Pi-sensitive mechanisms. The rise in plasma phosphate and/or the fall in plasma calcium may be (in)direct triggers affecting factors controlling glomerular filtration such as vascular tone of afferent and/or efferent arterioles where calcium channels play an important role [42]. PTH is also required for the downregulation of renal Pi transporters after 4 hours as this response was also blunted in PTX rats. Thus, our results demonstrate that PTH is required for the early response to high Pi intake.

We detected only small or no changes in FGF23, 1,25-$(OH)_2$-vitamin D_3 , insulin, and dopamine. The increase in dopamine or FGF23 was found only at the latest time point and is probably not responsible for the massive phosphaturia at earlier time points. In Pi infused intact rats, the late rise in plasma dopamine (but not in urine) may enhance PTH induced phosphaturia. Dopamine acts via D_1 receptors to downregulate NaPi-IIa in proximal tubules [9-11]. The increase in FGF23 in PTX rats occurs between 50 and 240 minutes after the Pi bolus (due to the small volumes of blood that could be collected only few FGF23 determinations were possible). This finding suggests first that in the absence of PTH, FGF23 levels adapt more acutely to the Pi overload, and second that systemic Pi can regulate FGF23 production and/or stability independently of PTH. Interestingly, FGF-23 production by osteocytes is not directly regulated by Pi [43] and instead requires previous production of PTH and activation of PKA and Wnt-pathways by PTHR1 [25, 44-45]

Intestinal Pi sensors and an intestine-derived phosphaturic factor had been postulated based on experiments with rats infused with Pi into duodenum. This

mechanism would allow for a rapid crosstalk between intestine and kidney and provide a feed-forward mechanism preventing a potentially detrimental hyperphosphatemia [24]. The existence of such feed-forward mechanisms has been demonstrated for potassium and salt [46-49]. However, Scanni et al had found in healthy humans that the rate of elimination and overall quantity of Pi excretion did not depend on the route of application (i.v. versus intraduodenal infusion)[25]. Consistently, our data do not provide any evidence for a role of the intestine in promoting phosphaturia. Moreover, in the absence of PTH, no phosphaturia could be elicited by intragastric Pi infusions.

The cumulative urinary elimination of the Pi bolus reached only about 50-60 % after 4 hours in intact animals whereas normophosphatemia was achieved much faster suggesting that a large amount of Pi had been either eliminated by other routes (i.e intestinal tract), or extravasated and accumulated in tissues, or complexed in blood into a pool that is not measurable by the Fiske-Subbarow method. Scanni *et al* showed that urinary excretion of Pi accounted for 100% of the intravenous Pi overload in humans, but full elimination required 120 hours [25]; the authors rule out a contribution of the gastrointestinal tract in the elimination of Pi. We quantified the Pi content in femurs as well as liver and skeletal muscle. Pi content in bones is much higher than in the other two organs and the measured values were so high that a rough estimation of the total amount of Pi stored in the skeleton (assuming a 10% contribution to body weight and a comparable composition of all bones) suggests that even if all the non-excreted Pi would have been accumulated in bones this would only result in a small change of content (~0.5%), non-detectable with the Fiske-Subbarow method. Although similar estimations predicted that partial accumulation of Pi in liver and skeletal muscle could be detectable, we failed to observe any changes. Thus, other approaches should be used in order to identify the organ/s responsible for a transient accumulation of a large excess of Pi.

In summary, our data indicate that: a) normophosphatemia is rapidly re-established after intravenous and intragastric Pi loading by mechanisms largely depending on the ability of the kidneys to excrete Pi, b) an efficient phosphaturic response requires increased levels of PTH and reduced expression of renal Pi

transporters, c) these compensatory responses are, to a major extent, similar whether or no Pi bypasses the gastrointestinal tract, and that d) reduced plasma Ca^{2+} together with elevated Pi may trigger the secretion of PTH in intravenously loaded animals. Our findings leave two major issues unresolved, namely the identity of the compartment responsible for the rapid “quenching” of a large fraction of the loaded Pi, and the nature of the signal that triggers the stimulation of PTH release.

CONCISE METHODS

Animal Experimental Protocol

Male Wistar rats (Charles River, Germany) weighing 250 - 350 g were adapted to a low Pi diet (0.1%) for 5 days. After an overnight fast in metabolic cages with free access to water, animals were anesthetized with 3% isoflurane/air, placed on a heated pad to maintain body temperature at 37 to 38°C. Rats inhaled constantly a low dose of anesthesia (1-2% isoflurane/air) until the end of the experiment. Catheters (BPE-T 50, Instech) were placed into the femoral vein, femoral artery, and urinary bladder (for infusion of solutions and collection of blood and urine, respectively). A Ringer's solution (116 mM NaCl, 1.2 mM KCl, 1 mM $CaCl_2$ and 2.7 mM $NaHCO_3$) containing 5 % glucose was infused continuously at a rate of 3.0 ml /hr until the end of the experiment. To determine baseline values, blood and urine samples were collected over a period of 30 minutes after the start of the Ringer infusion. In the infusion protocol, one milliliter of either Na_2HPO_4/NaH_2PO_4 (500, 150 or 50 mM, pH 7.4) or NaCl (500 or 150 mM, pH 7.4) was infused immediately after the baseline sampling within 2-3 minutes. For gavage, one milliliter of either 500 mM NaH_2PO_4/Na_2HPO_4 (pH 5) or NaCl (500 or 150 mM, pH 5) was administered directly into the stomach by using a gavage tube (FTP-15-100, Instech). Blood and urine samples were collected at different time intervals of post infusion or gavage. At the end of sample collections (40 min or 4 hrs), the animals were sacrificed and blood, kidneys, intestine (duodenum, jejunum, and ileum), liver, muscle and femur were harvested. Blood samples (both from intermediate time points and at termination) were centrifuged immediately upon collection, and plasma was aliquoted. Plasma and organs were store at -80°C until further used.

In addition, the identical experimental protocol to the one described above was performed in 350-450 g parathyrodectomized (PTX) male rats (Charles River) receiving 1% calcium gluconate in drinking water.

All animal experiments were according to Swiss and international laws of animal protection and all protocols were approved by the appropriate local Veterinary Authority (Kantonales Veterinäramt Zürich).

General analytical measurements

The concentration of Pi in plasma and urine was determined according to the Fiske & Subbarow method (Randox). Plasma and urinary levels of calcium were measured using a Quantichrom calcium kit from Bioassay system. The concentration of creatinine in plasma was enzymatically measured using a peroxidase-antiperoxidase (PAP)-creatinine kit (Biomed), whereas urinary creatinine was measured according to the Jaffe method. Fractional excretion of phosphate (FE(Pi)% was calculated using the formula: [urine phosphate x plasma creatinine] x100 / [plasma phosphate x urine creatinine]. The creatinine clearance was estimated from plasma and urinary creatinine concentrations and urine volume.

The concentration of glucose in plasma was measured by using Accu-Chek (Roche). Enzyme-linked immunosorbent assays (ELISA) kits were used to measure the concentrations in plasma and/or of intact PTH (Immutopic), intact FGF 23 (Kainos), insulin (Crystal Chem Inc) and dopamine (LDN). Plasma 1,25-$(OH)_2$ Vitamin D_3 was determined with a radioimmunoassy (RIA) kit from IDS. Protein concentrations in renal BBM were determined with the Bio-Rad protein assay (Bio-Rad Laboratories).

Isolation of renal brush border membrane vesicles (BBMV) and flux measurements of ^{32}Pi and ^{3}H-glucose

Kidney cortex and medulla were dissected from frozen kidneys and upon homogenization in a buffer containing 300 mM Mannitol, 5 EGTA and 12 Tris-HCl, pH 7.1, BBMV were isolated according to the Mg^{2+}-precipitation method, as described in detail [50]. Uptake of ^{32}Pi and ^{3}H-glucose were measured in three different solutions, all three containing 300 mM mannitol plus 20 mM HEPES-Tris, pH 7.4 and either 125 mM NaCl, 125 mM KCl or 125 mM NaCl ~~plus 7.5 mM phosphonoformic acid (PFA)~~. The uptake solutions contained either 0.125 μM K_2HPO4/KH_2PO_4, pH 7.4 as cold substrate

and ^{32}Pi as a tracer, or 0.125 μM D-glucose as cold substrate and ^{3}H-D-glucose as tracer. To measure the incorporation of ^{32}Pi /^{3}H-glucose, 10 μl of freshly prepared BBMV were incubated for 1 minute or 2 hours with 40 μl of the different uptake solutions. The 1 min time point was chosen as ^{32}Pi/^{3}H-glucose uptake was in the linear phase of the maximal transport rate as determined earlier [50]. After the indicated incubation time, uptakes were stopped by transferring 20 μl of sample to 1 ml of ice cold stop solution (100 mM Mannitol, 5 mM Tris-HCl, 150 mM NaCl, 5 mM Pi, 5 mM glucose). The resulting suspension was then spotted onto a filter and vacuum- washed with 10 ml of ice cold stop solution. Filters were finally transferred into plastic vials and upon addition of 3 ml scintillation medium (Perkin Elmer) the retained radioactivity was measured on a β-counter (Packard, TRI-CARB 2900TR). All measurements were carried out in triplicate. The Na^+ dependent uptakes were calculated by subtracting the uptake values obtained in the K^+-medium from those measured in the Na^+-medium (total uptake). Since PFA is a competitive inhibitor of SLC34 cotransporters (NaPi-IIa and NaPi-IIc), SLC34 mediated uptake was determined by subtracting the uptake values obtained in the presence of PFA from the Na^+ dependent values. The remaining BBMV that were not used in the uptake experiments were stored immediately at -80 ^{0}C for further experiments.

Immunoblotting

The protein expression levels of NaPi-IIa and NaPi-IIc in renal BBM were quantified by immunoblotting. To this end, 20 μg of BBMs were solubilized in Laemmli's buffer and separated on 10% SDS-PAGE, and then transferred to polyvinylidene difluoride membranes (Millipore). After blocking non-specific binding with 5 % milk powder in Tris-buffered saline containing 0.1% tween-20 for 40 min, the blots were incubated overnight at 4 ^{0}C with primary antibodies against NaPi-IIa (1: 4000)[51], NaPi-IIc (1: 2500)[52-53] and β-Actin (1:10000, Sigma Aldrich, Buchs, Switzerland). After washing and further blocking, blots were incubated with appropriate secondary antibodies for 2 hours at room temperature. Finally, membranes were exposed to chemiluminescent substrate for 5 minutes and protein signals were detected on a LAS-4000 luminescent image

analyzer (Fujifilm). All the images were quantified with Advanced Image Data Analyzer (Raytest). The expression of both cotransporters was normalized to the abundance of β-Actin.

Determination of Phosphate in tissues

Tissues (liver, skeletal muscle, and femur) collected from intact and parathyroidectomized rats 4 hours after infusion or gavage were dried in an oven at 70^0C for 24 hours. Samples were weighed, transferred into a silica crucible and burned to ashes in an electric furnace at 700^0C for 12 hours. 1N HCl was used to dissolve the Pi present in ashes and after centrifugation supernatants were collected for Pi determination by the above mentioned Fiske & Subbarow method.

Statistical analysis

Statistical significances were calculated by student's t-test or one-way ANOVA (Bonferroni), as indicated. $P < 0.05$ was considered significant. Results are presented as mean ± SEM.

Acknowledgments

The authors would like to acknowledge the technical support by Udo Schnitzbauer as well of the ZIRP Core Facility for Rodent Phenotyping. The study was supported by a grant from the National Center for Competence in Research NCCR Kidney.CH, Switzerland to J. Biber and C.A. Wagner.

Statement of competing financial interests

The authors declare that they have no competing financial interests. N. Hernando and C.A. Wagner received financial research support from Astra Zeneca unrelated to this project.

FIGURE LEGENDS

Figure 1. Effect of intravenous and intragastric administration of phosphate on body phosphate distribution and renal excretion in intact rats

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma Pi, (**B**) urinary Pi/creatinine, (**C**) creatinine clearance, and (**D**) cumulative Pi excretion. **(E)** concentration of Pi in femur, (**F**) concentration of Pi in skeletal muscle, (**G**) concentration of Pi in liver. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 or ***P* <0.01 or *** *P*< 0.001 vs. *time 0*, Anova test, n = 5-9/group and time point.

Figure 2. Effect of intravenous and intragastric administration of phosphate on Ca^{2+} plasma levels and urinary excretion in intact rats

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma Ca^{2+}, (**B**) urinary Ca^{2+}/ creatinine. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*, Anova test, n = 5-9/group and time point.

Figure 3. Effect of intravenous and intragastric administration of phosphate on hormonal levels in intact rats

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma intact Parathyroid hormone (PTH), (**B**) plasma intact FGF23, (**C**) plasma 1,25-$(OH)_2$ vitamin D_3, (**D**) plasma insulin, (**E**) plasma dopamine, and (F) urine dopamine. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*, Anova test, n = 5-9/group and time point.

Figure 4. **Effect of intravenous and intragastric administration of phosphate on phosphate and glucose transport activities in renal brush border membrane vesicles from intact rats**

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. Kidneys were extracted 40 minutes or 4 hours after infusion or gavage~~post administration~~ and Na^+-dependent and -independent Pi and glucose transport activities measured. Pi transport was assayed in the presence or absence of the SLC34 transport inhibitor phosphonoformic acid (PFA). (**A**) ^{32}P-uptakes 40 minutes after intravenous infusion of saline (grey bars) and Pi (black bars), (**B**) ^{32}P-uptakes 40 minutes after intragastric administration of saline (grey bars) and Pi (black bars), (**C**) ^{32}P-uptakes 4 hours after intravenous infusion of saline (grey bars) and Pi (black bars), (**D**) ^{32}P-uptakes 4 hours after intragastric administration of saline (grey bars) and Pi (black bars), (**E**) ^{3}H-D-glucose uptakes 40 minutes after intravenous and intragastric administration of saline (grey bars) and Pi (black bars), (**F**) ^{3}H-D-glucose uptakes 4 hours after intravenous and intragastric administration of saline (grey bars) and Pi (black bars). Data are presented as the mean ± SEM. *P< 0.05 versus saline group, Unpaired Student's t-test, n = 5-9/group and time point.

.

Figure 5. **Effect of intravenous and intragastric administration of phosphate on the expression of renal Na/Pi-cotransporters in intact rats**

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. Kidneys were extracted 40 minutes or 4 hours post administration and the abundance of NaPi-IIa and NaPi-IIc in BBM was determined by Western blot. Expression of cotransporters (**A**) 40 minutes after intravenous infusion of saline (grey bars) and Pi (black bars), (**B**) 40 minutes after intragastric administration of saline (grey bars) and Pi (black bars), (**C**) 4 hours after intravenous infusion of saline (grey bars) and Pi (black bars), (**B**) 4 hours after intragastric administration of saline (grey bars) and Pi (black bars). *P< 0.05 versus saline group, Unpaired Student's t-test, n = 5-9/group and time point.

Figure 6. **Effect of intravenous and intragastric administration of phosphate on ~~body phosphate distribution and~~ renal excretion in parathyroidectomized rats**

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma Pi, (**B**) urinary Pi/creatinine, (**C**) creatinine clearance, and (**D**) ~~ac~~cumulative Pi excretion. ~~**(E)** concentration of Pi in femur, (**F**) concentration of Pi in skeletal muscle, (**G**) concentration of Pi in liver.~~ Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 or ***P* <0.01 vs. *time 0*, Anova test, n = 4-6/group and time point.

Figure 7. **Effect of intravenous and intragastric administration of phosphate on Ca^{2+} plasma levels and urinary excretion in parathyroidectomized rats**

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma Ca^{2+}, (**B**) urinary Ca^{2+}/creatinine. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*, Anova test, n = 4-6/group and time point.

Figure 8. **Effect of intravenous and intragastric administration of phosphate on hormonal levels in parathyroidectomized rats**

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) plasma intact PTH, (**B**) plasma intact FGF23, (**C**) plasma 1,25-$(OH)_2$ vitamin D_3, (**D**) plasma insulin, (**E**) plasma dopamine, and (F) urine dopamine. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*, Anova test, n = 4-6/group and time point.

Figure 9. **Effect of intravenous and intragastric administration of phosphate on phosphate and glucose transport activities in renal brush border membrane vesicles from parathyroidectomized rats**

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. Kidneys were extracted 4 hours after infusion or gavage~~post administration~~ and Na^+-dependent and -independent Pi and glucose transport activities in isolated brush border membrane vesicles measured. Pi transport was assayed in the presence or absence of the SLC34 transport inhibitor phosphonoformic acid (PFA). (**A**) ^{32}P-uptakes 4 hours after intravenous infusion of saline (grey bars) and Pi (black bars), (**B**) ^{32}P-uptakes 4 hours after intragastric administration of saline (grey bars) and Pi (black bars), (**C**) ^{3}H-D-glucose uptakes 4 hours after intravenous and intragastric administration of saline (grey bars) and Pi (black bars). Data are presented as the mean ± SEM. *P< 0.05 versus saline group, unpaired Student's t-test, n = 4-6/group and time point.

Figure 10. **Effect of intravenous and intragastric administration of phosphate on the expression of renal Na/Pi-cotransporters in parathyroidectomized rats**

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. Kidneys were extracted 4 hours post administration and the abundance of NaPi-IIa and NaPi-IIc in BBM was determined by Western blot. Expression of cotransporters (**A**) 4 hours after intravenous infusion of saline (grey bars) and Pi (black bars), (**B**) 4 hours after intragastric administration of saline (grey bars) and Pi (black bars). *P< 0.05 versus saline group, unpaired Student's t-test, n = 4-6/group and time point.

REFERENCES

1. Wagner, CA, Hernando, N, Forster, IC, Biber, J: The SLC34 family of sodium-dependent phosphate transporters. *Pflugers Archiv : European journal of physiology,* 466**:** 139-153, 2014.

2. Biber, J, Hernando, N, Forster, I: Phosphate transporters and their function. *Annu Rev Physiol,* 75**:** 535-550, 2013.

3. Bergwitz, C, Juppner, H: Regulation of Phosphate Homeostasis by PTH, Vitamin D, and FGF23. *Annu Rev Med,* 61**:** 91-104, 2010.

4. Berndt, T, Kumar, R: Novel mechanisms in the regulation of phosphorus homeostasis. *Physiology (Bethesda),* 24**:** 17-25, 2009.

5. Biber, J, Hernando, N, Forster, I: Phosphate Transporters and Their Function. *Annual Review of Physiology, Vol 75,* 75**:** 535-550, 2013.

6. Hu, MC, Shiizaki, K, Kuro-o, M, Moe, OW: Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol,* 75**:** 503-533, 2013.

7. Martin, A, David, V, Quarles, LD: Regulation and function of the FGF23/klotho endocrine pathways. *Physiol Rev,* 92**:** 131-155, 2012.

8. Bergwitz, C, Juppner, H: Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu Rev Med,* 61**:** 91-104, 2010.

9. Weinman, EJ, Biswas, R, Steplock, D, Wang, P, Lau, YS, Desir, GV, Shenolikar, S: Increased renal dopamine and acute renal adaptation to a high-phosphate diet. *Am J Physiol Renal Physiol,* 300**:** F1123-1129, 2011.

10. Bacic, D, Capuano, P, Baum, M, Zhang, J, Stange, G, Biber, J, Kaissling, B, Moe, OW, Wagner, CA, Murer, H: Activation of dopamine D1-like receptors induces acute internalization of the renal Na+/phosphate cotransporter NaPi-IIa in mouse kidney and OK cells. *Am J Physiol Renal Physiol,* 288**:** F740-747, 2005.

11. Isaac, J, Berndt, TJ, Chinnow, SL, Tyce, GM, Dousa, TP, Knox, FG: Dopamine enhances the phosphaturic response to parathyroid hormone in phosphate-deprived rats. *J Am Soc Nephrol,* 2**:** 1423-1429, 1992.

12. Capuano, P, Radanovic, T, Wagner, CA, Bacic, D, Kato, S, Uchiyama, Y, St-Arnoud, R, Murer, H, Biber, J: Intestinal and renal adaptation to a low-Pi diet of type II NaPi cotransporters in vitamin D receptor- and 1alphaOHase-deficient mice. *Am J Physiol Cell Physiol,* 288**:** C429-434, 2005.

13. Marks, J, Debnam, ES, Unwin, RJ: Phosphate homeostasis and the renal-gastrointestinal axis. *Am J Physiol Renal Physiol,* 299**:** F285-296, 2010.

14. Marks, J, Srai, SK, Biber, J, Murer, H, Unwin, RJ, Debnam, ES: Intestinal phosphate absorption and the effect of vitamin D: a comparison of rats with mice. *Exp Physiol,* 91**:** 531-537, 2006.

15. Allon, M, Rodriguez, M, Llach, F: Insulin in the acute renal adaptation to dietary phosphate restriction in the rat. *Kidney international,* 37**:** 14-20, 1990.

16. Torres, PA, De Brauwere, DP: Three feedback loops precisely regulating serum phosphate concentration. *Kidney Int,* 80**:** 443-445, 2011.

17. Blaine, J, Chonchol, M, Levi, M: Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol,* 10**:** 1257-1272, 2015.

18. Rowe, PS: A unified model for bone-renal mineral and energy metabolism. *Curr Opin Pharmacol,* 22**:** 64-71, 2015.

19. Hu, MC, Shi, M, Zhang, J, Pastor, J, Nakatani, T, Lanske, B, Shawkat Razzaque, M, Rosenblatt, KP, Baum, MG, Kuro, OM, Moe, OW: Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J*, 2010.

20. Chang, Q, Hoefs, S, van der Kemp, AW, Topala, CN, Bindels, RJ, Hoenderop, JG: The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science,* 310**:** 490-493, 2005.

21. Berndt, TJ, Bielesz, B, Craig, TA, Tebben, PJ, Bacic, D, Wagner, CA, O'Brien, S, Schiavi, S, Biber, J, Murer, H, Kumar, R: Secreted frizzled-related protein-4 reduces sodium-phosphate co-transporter abundance and activity in proximal tubule cells. *Pflugers Arch,* 451**:** 579-587, 2006.

22. Marks, J, Churchill, LJ, Debnam, ES, Unwin, RJ: Matrix extracellular phosphoglycoprotein inhibits phosphate transport. *J Am Soc Nephrol,* 19**:** 2313-2320, 2008.

23. David, V, Martin, A, Hedge, AM, Rowe, PS: Matrix extracellular phosphoglycoprotein (MEPE) is a new bone renal hormone and vascularization modulator. *Endocrinology,* 150**:** 4012-4023, 2009.

24. Berndt, T, Thomas, LF, Craig, TA, Sommer, S, Li, X, Bergstralh, EJ, Kumar, R: Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *Proc Natl Acad Sci U S A,* 104**:** 11085-11090, 2007.

25. Scanni, R, vonRotz, M, Jehle, S, Hulter, HN, Krapf, R: The human response to acute enteral and parenteral phosphate loads. *Journal of the American Society of Nephrology : JASN,* 25**:** 2730-2739, 2014.

26. Almaden, Y, Hernandez, A, Torregrosa, V, Canalejo, A, Sabate, L, Fernandez Cruz, L, Campistol, JM, Torres, A, Rodriguez, M: High phosphate level directly stimulates parathyroid hormone secretion and synthesis by human parathyroid tissue in vitro. *J Am Soc Nephrol,* 9**:** 1845-1852, 1998.

27. Almaden, Y, Rodriguez-Ortiz, ME, Canalejo, A, Canadillas, S, Canalejo, R, Martin, D, Aguilera-Tejero, E, Rodriguez, M: Calcimimetics normalize the phosphate-induced stimulation of PTH secretion in vivo and in vitro. *J Nephrol,* 22**:** 281-288, 2009.

28. Hansch, E, Forgo, J, Murer, H, Biber, J: Role of microtubules in the adaptive response to low phosphate of Na/Pi cotransport in opossum kidney cells. *Pflügers Arch,* 422**:** 516-522, 1993.

29. Reshkin, SJ, Forgo, J, Biber, J, Murer, H: Functional assymetry of phosphate transport and its regulation in OK cells: phosphate adaptation. *Pflugers Arch,* 419**:** 256-262, 1991.

30. Camalier, CE, Yi, M, Yu, LR, Hood, BL, Conrads, KA, Lee, YJ, Lin, Y, Garneys, LM, Bouloux, GF, Young, MR, Veenstra, TD, Stephens, RM, Colburn, NH, Conrads, TP, Beck, GR, Jr.: An integrated understanding of the physiological response to elevated extracellular phosphate. *J Cell Physiol,* 228**:** 1536-1550, 2013.

31. Khoshniat, S, Bourgine, A, Julien, M, Weiss, P, Guicheux, J, Beck, L: The emergence of phosphate as a specific signaling molecule in bone and other cell types in mammals. *Cell Mol Life Sci,* 68**:** 205-218, 2011.

32. Michigami, T: Extracellular phosphate as a signaling molecule. *Contrib Nephrol,* 180**:** 14-24, 2013.

33. Cuche, JL, Marchand, GR, Greger, RF, Lang, RC, Knox, FG: Phosphaturic effect of dopamine in dogs. Possible role of intrarenally produced dopamine in phosphate regulation. *J Clin Invest,* 58**:** 71-76, 1976.

34. Nishida, Y, Taketani, Y, Yamanaka-Okumura, H, Imamura, F, Taniguchi, A, Sato, T, Shuto, E, Nashiki, K, Arai, H, Yamamoto, H, Takeda, E: Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. *Kidney Int,* 70**:** 2141-2147, 2006.

35. Martin, DR, Ritter, CS, Slatopolsky, E, Brown, AJ: Acute regulation of parathyroid hormone by dietary phosphate. *Am J Physiol Endocrinol Metab,* 289**:** E729-734, 2005.

36. Bourgeois, S, Capuano, P, Stange, G, Muhlemann, R, Murer, H, Biber, J, Wagner, CA: The phosphate transporter NaPi-IIa determines the rapid renal adaptation to dietary phosphate intake in mouse irrespective of persistently high FGF23 levels. *Pflugers Arch,* 465**:** 1557-1572, 2013.

37. Berndt, T, Thomas, LF, Craig, TA, Sommer, S, Li, X, Bergstralh, EJ, Kumar, R: Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *Proceedings of the National Academy of Sciences of the United States of America,* 104**:** 11085-11090, 2007.

38. Zajicek, HK, Wang, H, Puttaparthi, K, Halaihel, N, Markovich, D, Shayman, J, Beliveau, R, Wilson, P, Rogers, T, Levi, M: Glycosphingolipids modulate renal phosphate transport in potassium deficiency. *Kidney Int,* 60**:** 694-704, 2001.

39. Weinman, EJ, Biswas, R, Steplock, D, Douglass, TS, Cunningham, R, Shenolikar, S: Sodium-hydrogen exchanger regulatory factor 1 (NHERF-1) transduces signals that mediate dopamine inhibition of sodium-phosphate co-transport in mouse kidney. *J Biol Chem,* 285**:** 13454-13460, 2010.

40. Weinman, EJ, Biswas, RS, Peng, G, Shen, L, Turner, CL, E, X, Steplock, D, Shenolikar, S, Cunningham, R: Parathyroid hormone inhibits renal phosphate transport by phosphorylation of serine 77 of sodium-hydrogen exchanger regulatory factor-1. *J Clin Invest,* 117**:** 3412-3420, 2007.

41. Almaden, Y, Canalejo, A, Hernandez, A, Ballesteros, E, Garcia-Navarro, S, Torres, A, Rodriguez, M: Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *J Bone Miner Res,* 11**:** 970-976, 1996.

42. Hansen, PB: Functional and pharmacological consequences of the distribution of voltage-gated calcium channels in the renal blood vessels. *Acta Physiol (Oxf),* 207**:** 690-699, 2013.

43. Liu, S, Zhou, J, Tang, W, Jiang, X, Rowe, DW, Quarles, LD: Pathogenic role of Fgf23 in Hyp mice. *American journal of physiology Endocrinology and metabolism,* 291**:** E38-49, 2006.

44. Lavi-Moshayoff, V, Wasserman, G, Meir, T, Silver, J, Naveh-Many, T: PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *American journal of physiology Renal physiology,* 299**:** F882-889, 2010.

45. Rhee, Y, Allen, MR, Condon, K, Lezcano, V, Ronda, AC, Galli, C, Olivos, N, Passeri, G, O'Brien, CA, Bivi, N, Plotkin, LI, Bellido, T: PTH receptor signaling in osteocytes governs periosteal bone formation and intracortical remodeling. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research,* 26**:** 1035-1046, 2011.

46. Youn, JH, McDonough, AA: Recent advances in understanding integrative control of potassium homeostasis. *Annu Rev Physiol,* 71**:** 381-401, 2009.

47. Preston, RA, Afshartous, D, Rodco, R, Alonso, AB, Garg, D: Evidence for a gastrointestinal-renal kaliuretic signaling axis in humans. *Kidney Int,* 88**:** 1383-1391, 2015.

48. Mueller, T, Dieplinger, B: The guanylin peptide family and the proposed gastrointestinal-renal natriuretic signaling axis. *Kidney Int,* 82**:** 1253-1255, 2012.

49. Michell, AR, Debnam, ES, Unwin, RJ: Regulation of renal function by the gastrointestinal tract: potential role of gut-derived peptides and hormones. *Annu Rev Physiol,* 70**:** 379-403, 2008.

50. Biber, J, Stieger, B, Stange, G, Murer, H: Isolation of renal proximal tubular brush-border membranes. *Nature protocols,* 2**:** 1356-1359, 2007.

51. Custer, M, Lötscher, M, Biber, J, Murer, H, Kaissling, B: Expression of Na-P_i cotransport in rat kidney: localization by RT-PCR and immunohistochemistry. *Am J Physiol,* 266**:** F767-774, 1994.

52. Nowik, M, Picard, N, Stange, G, Capuano, P, Tenenhouse, HS, Biber, J, Murer, H, Wagner, CA: Renal phosphaturia during metabolic acidosis revisited: molecular mechanisms for decreased renal phosphate reabsorption. *Pflugers Arch,* 457**:** 539-549, 2008.

53. Hernando, N, Myakala, K, Simona, F, Knopfel, T, Thomas, L, Murer, H, Wagner, CA, Biber, J: Intestinal Depletion of NaPi-IIb/Slc34a2 in Mice: Renal and Hormonal Adaptation. *J Bone Miner Res,* 30**:** 1925-1937, 2015.

Figure 1

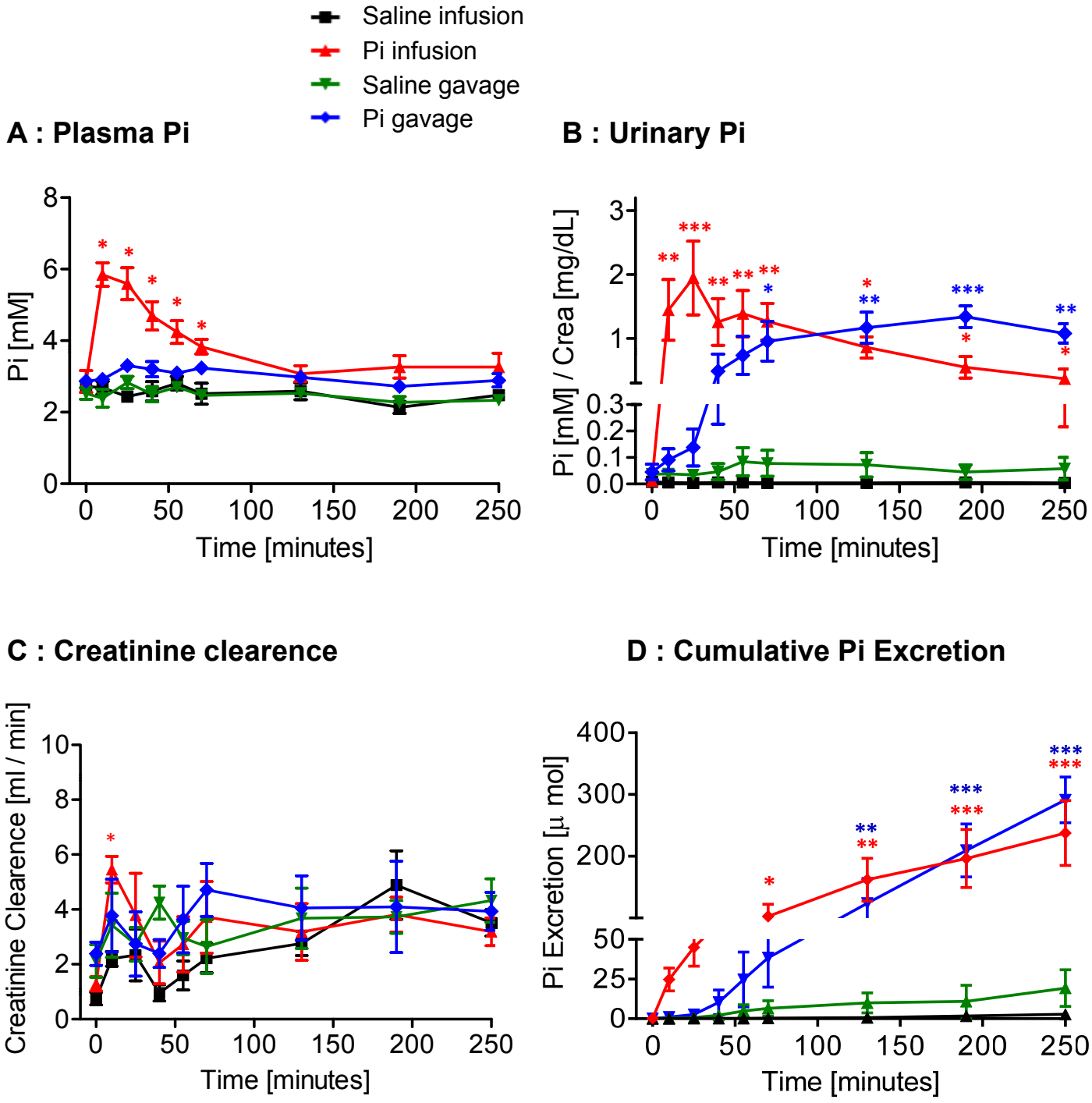

Saline infusion
Pi infusion
Saline gavage
Pi gavage
A : Plasma Pi
Pi [mM]
Time [minutes]
B : Urinary Pi
Pi [mM] / Crea [mg/dL]
Time [minutes]
C : Creatinine clearence
Creatinine Clearence [ml / min]
Time [minutes]
D : Cumulative Pi Excretion
Pi Excretion [µ mol]
Time [minutes]

Figure 2

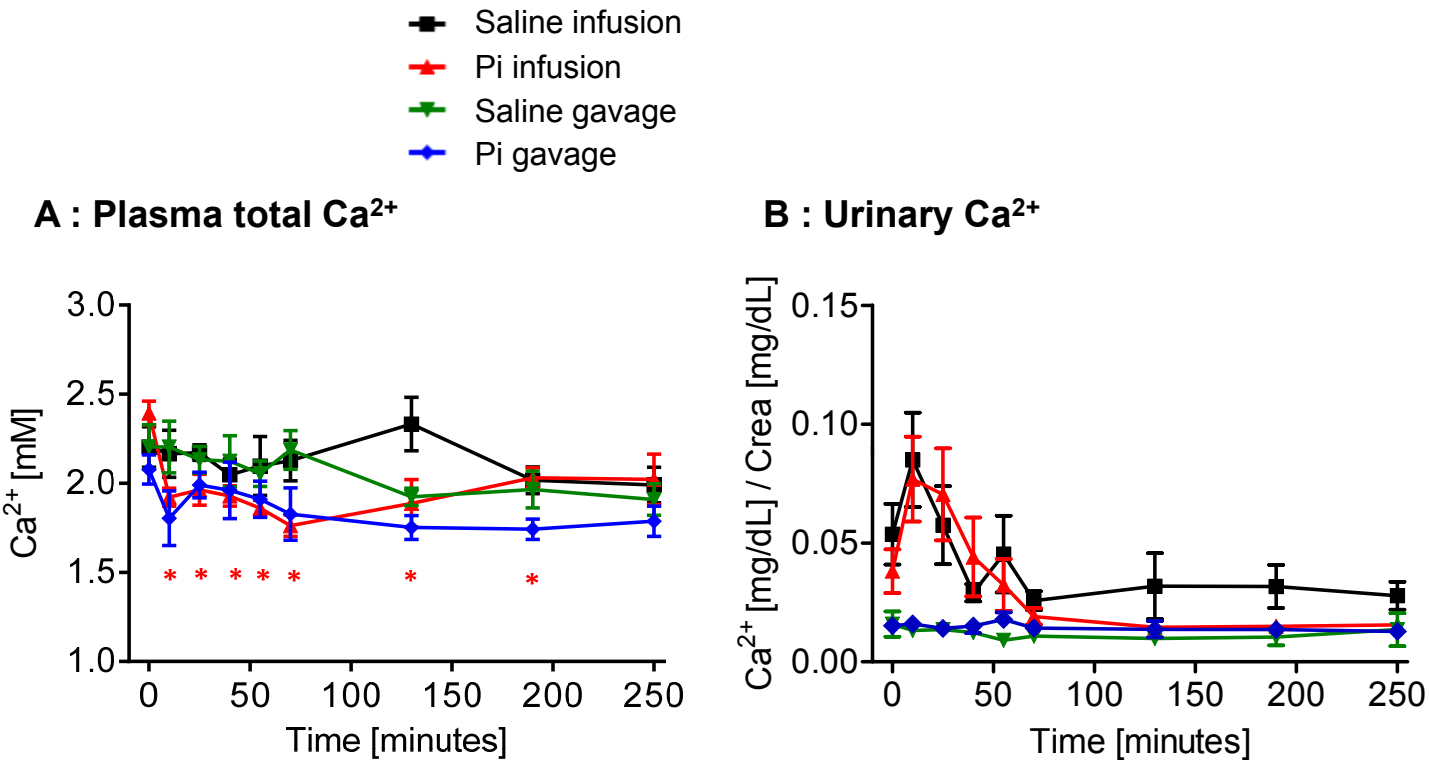

Saline infusion
Pi infusion
Saline gavage
Pi gavage
A : Plasma total Ca2+
Ca2+ [mM]
3.0
2.5
2.0
1.5
1.0
0
50
100
150
200
250
Time [minutes]
B : Urinary Ca2+
Ca2+ [mg/dL] / Crea [mg/dL]
0.15
0.10
0.05
0.00
0
50
100
150
200
250
Time [minutes]

Figure 3

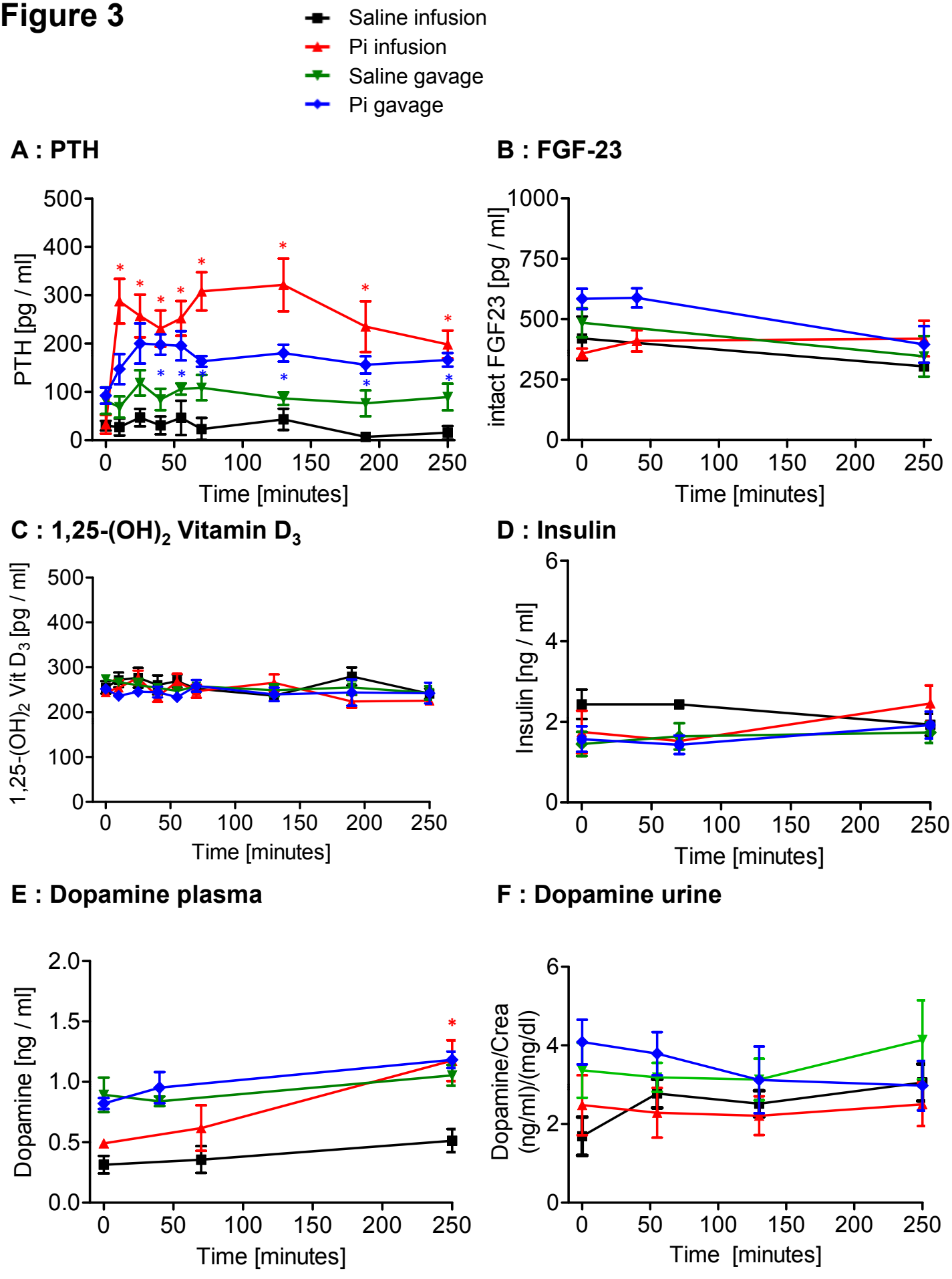

Saline infusion
Pi infusion
Saline gavage
Pi gavage
A : PTH
PTH [pg / ml]
Time [minutes]
B : FGF-23
intact FGF23 [pg / ml]
Time [minutes]
C : 1,25-(OH)2 Vitamin D3
1,25-(OH)2 Vit D3 [pg / ml]
Time [minutes]
D : Insulin
Insulin [ng / ml]
Time [minutes]
E : Dopamine plasma
Dopamine [ng / ml]
Time [minutes]
F : Dopamine urine
Dopamine/Crea (ng/ml)/(mg/dl)
Time [minutes]

Figure 4

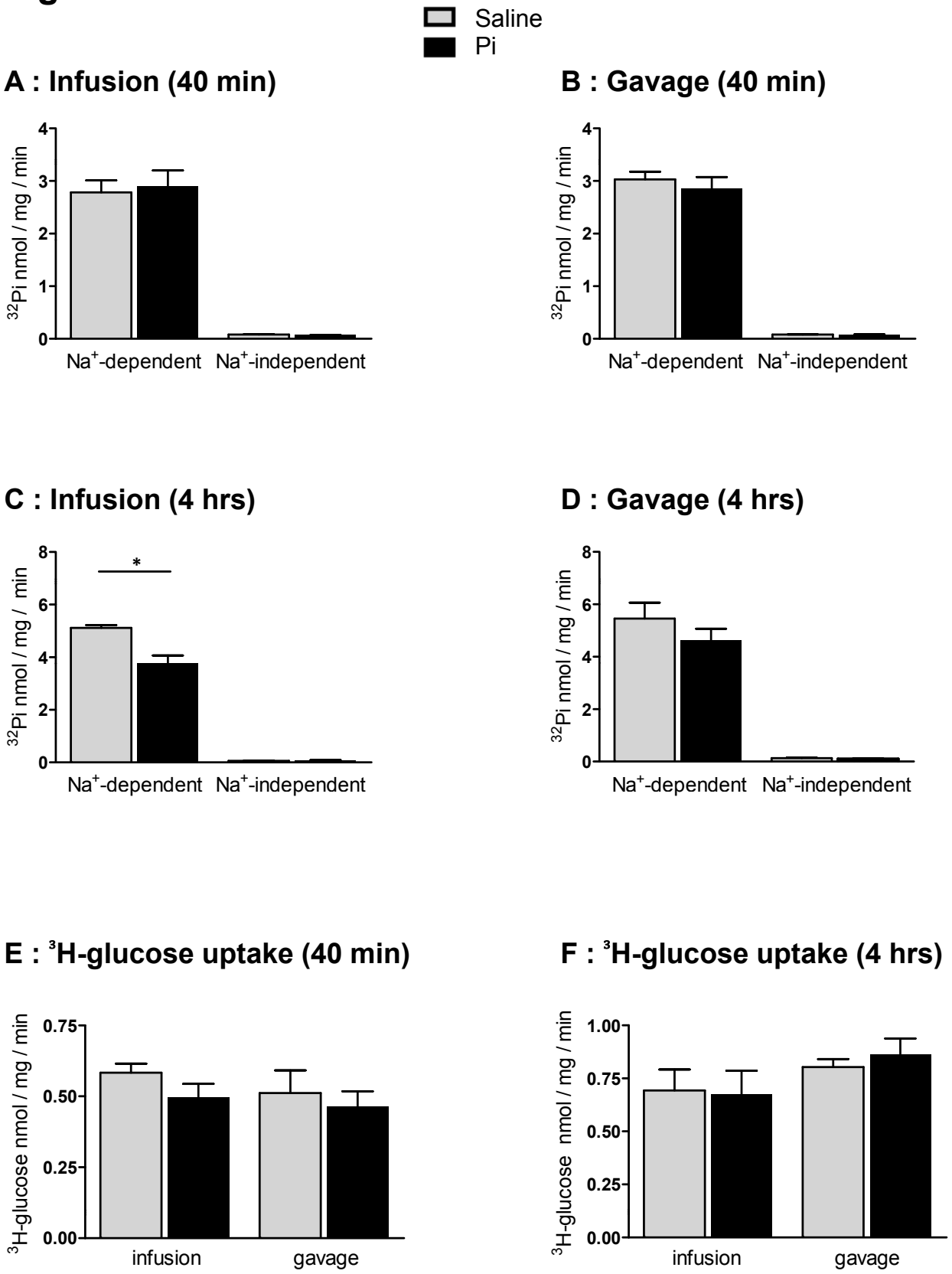
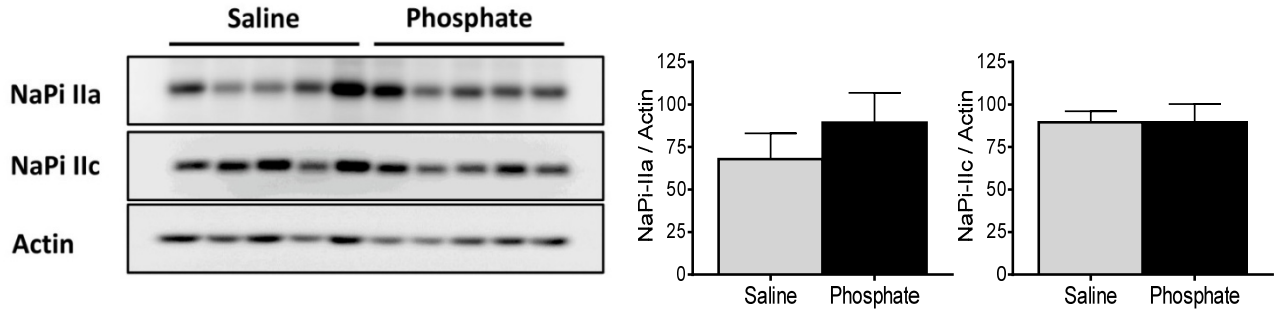
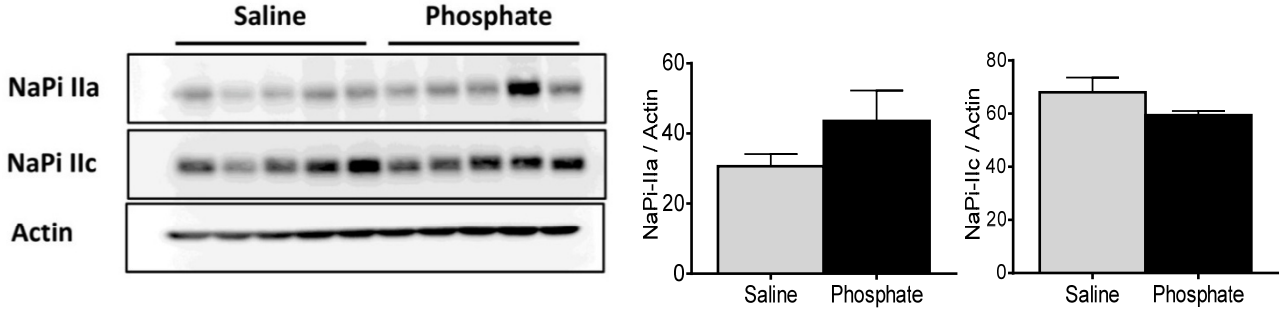

Saline
Pi
A : Infusion (40 min)
B : Gavage (40 min)
C : Infusion (4 hrs)
D : Gavage (4 hrs)
E : 3H-glucose uptake (40 min)
F : 3H-glucose uptake (4 hrs)
32Pi nmol / mg / min
3H-glucose nmol / mg / min
Na+-dependent
Na+-independent
infusion
gavage
*

Figure 5

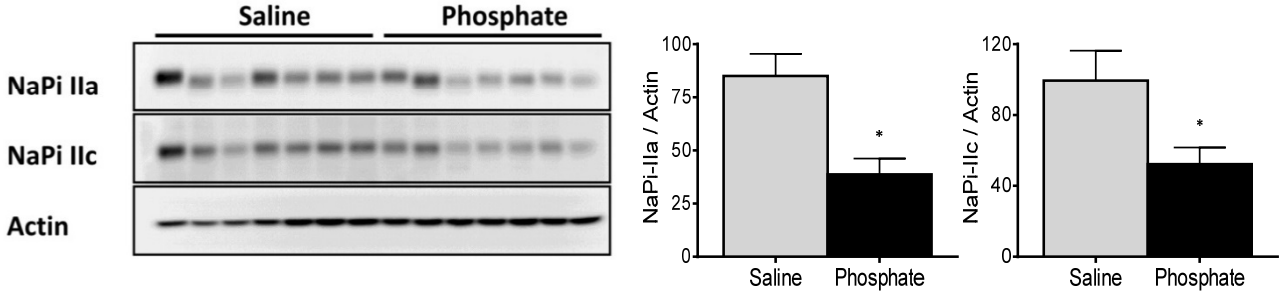
A : Infusion (40 min)



B : Gavage (40 min)



C : Infusion (4 hrs)



D : Gavage (4 hrs)

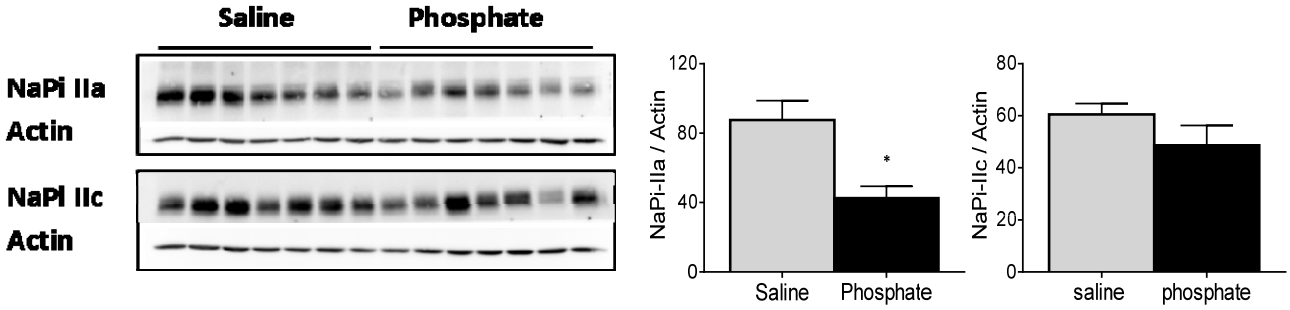


Figure 6

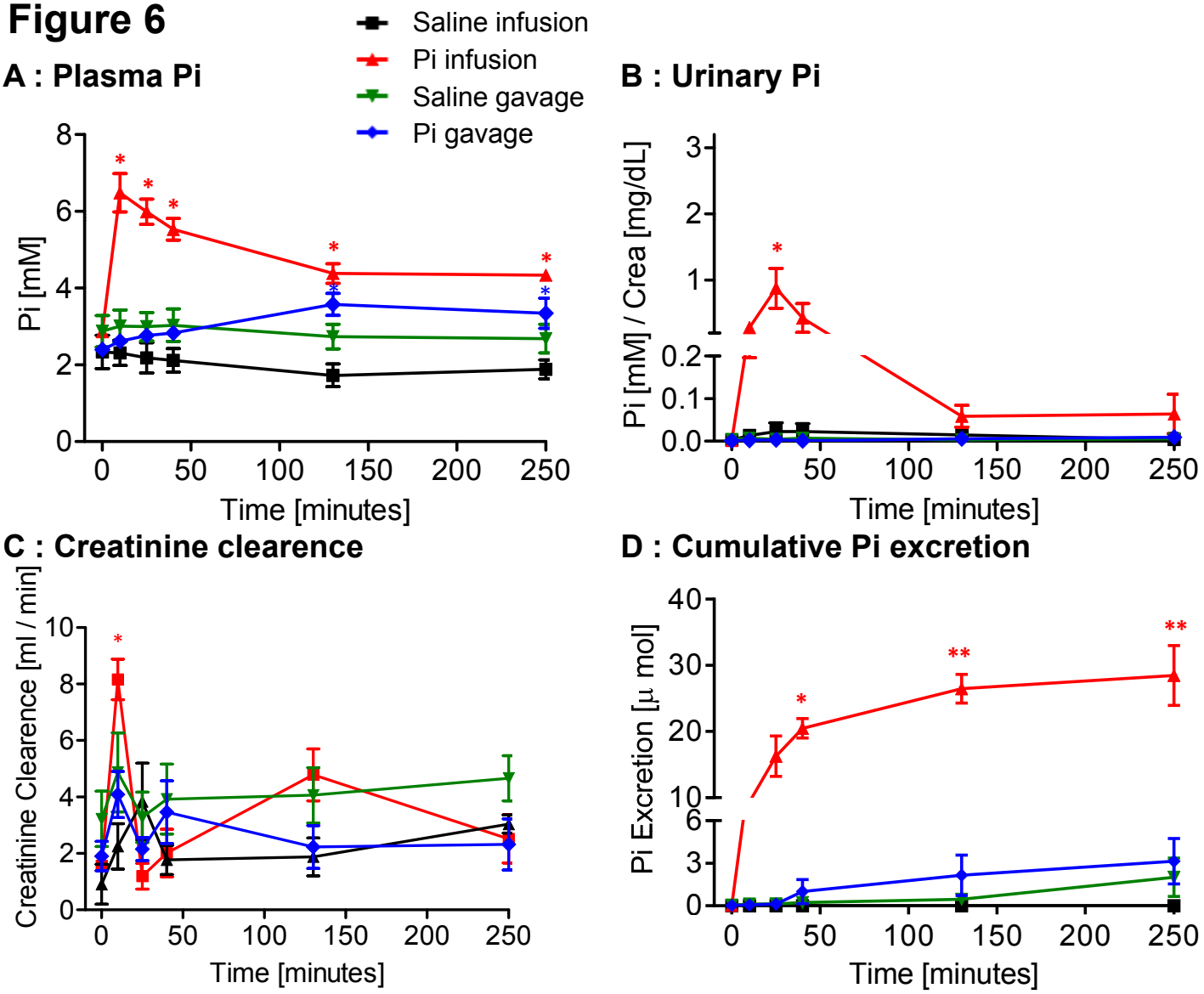

Saline infusion
Pi infusion
Saline gavage
Pi gavage
A : Plasma Pi
Pi [mM]
Time [minutes]
B : Urinary Pi
Pi [mM] / Crea [mg/dL]
Time [minutes]
C : Creatinine clearence
Creatinine Clearence [ml / min]
Time [minutes]
D : Cumulative Pi excretion
Pi Excretion [µ mol]
Time [minutes]

Figure 7

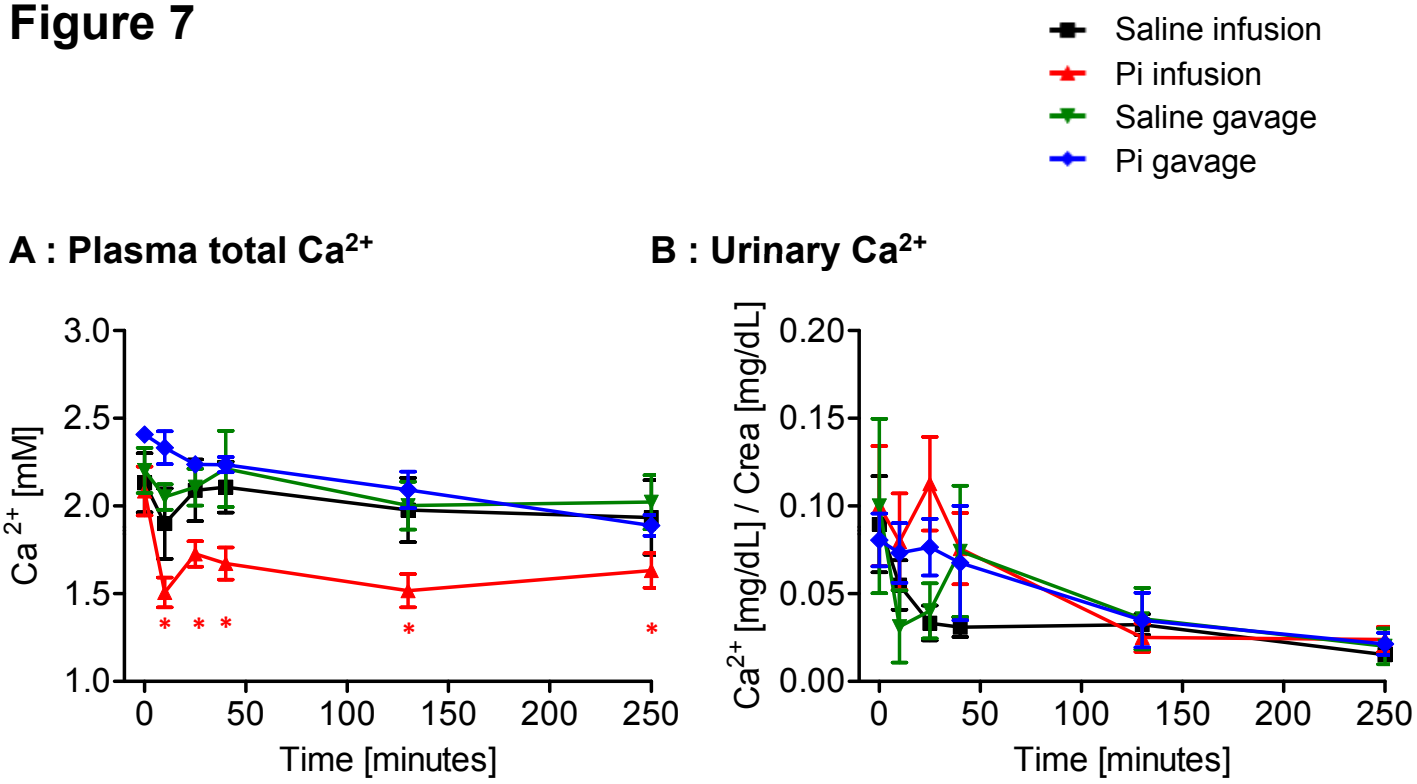

Saline infusion
Pi infusion
Saline gavage
Pi gavage
A : Plasma total Ca2+
B : Urinary Ca2+
Ca 2+ [mM]
3.0
2.5
2.0
1.5
1.0
0
50
100
150
200
250
Time [minutes]
Ca2+ [mg/dL] / Crea [mg/dL]
0.20
0.15
0.10
0.05
0.00
0
50
100
150
200
250
Time [minutes]

Figure 8

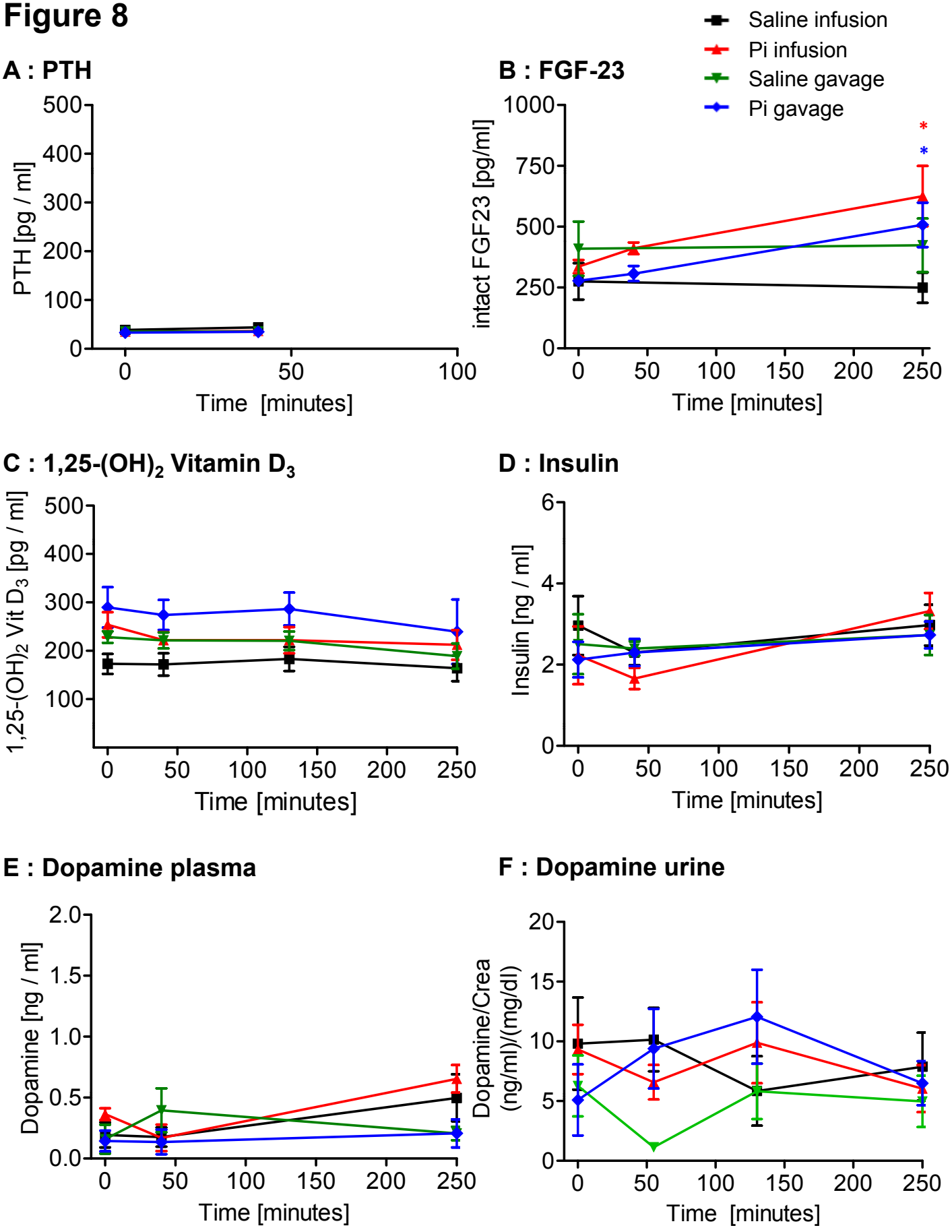

A : PTH
PTH [pg / ml]
500
400
300
200
100
0
0
50
100
Time [minutes]
B : FGF-23
Saline infusion
Pi infusion
Saline gavage
Pi gavage
intact FGF23 [pg/ml]
1000
750
500
250
0
*
*
0
50
100
150
200
250
Time [minutes]
C : 1,25-(OH)2 Vitamin D3
1,25-(OH)2 Vit D3 [pg / ml]
500
400
300
200
100
0
50
100
150
200
250
Time [minutes]
D : Insulin
Insulin [ng / ml]
6
4
2
0
0
50
100
150
200
250
Time [minutes]
E : Dopamine plasma
Dopamine [ng / ml]
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
Time [minutes]
F : Dopamine urine
Dopamine/Crea (ng/ml)/(mg/dl)
20
15
10
5
0
0
50
100
150
200
250
Time [minutes]

Figure 9

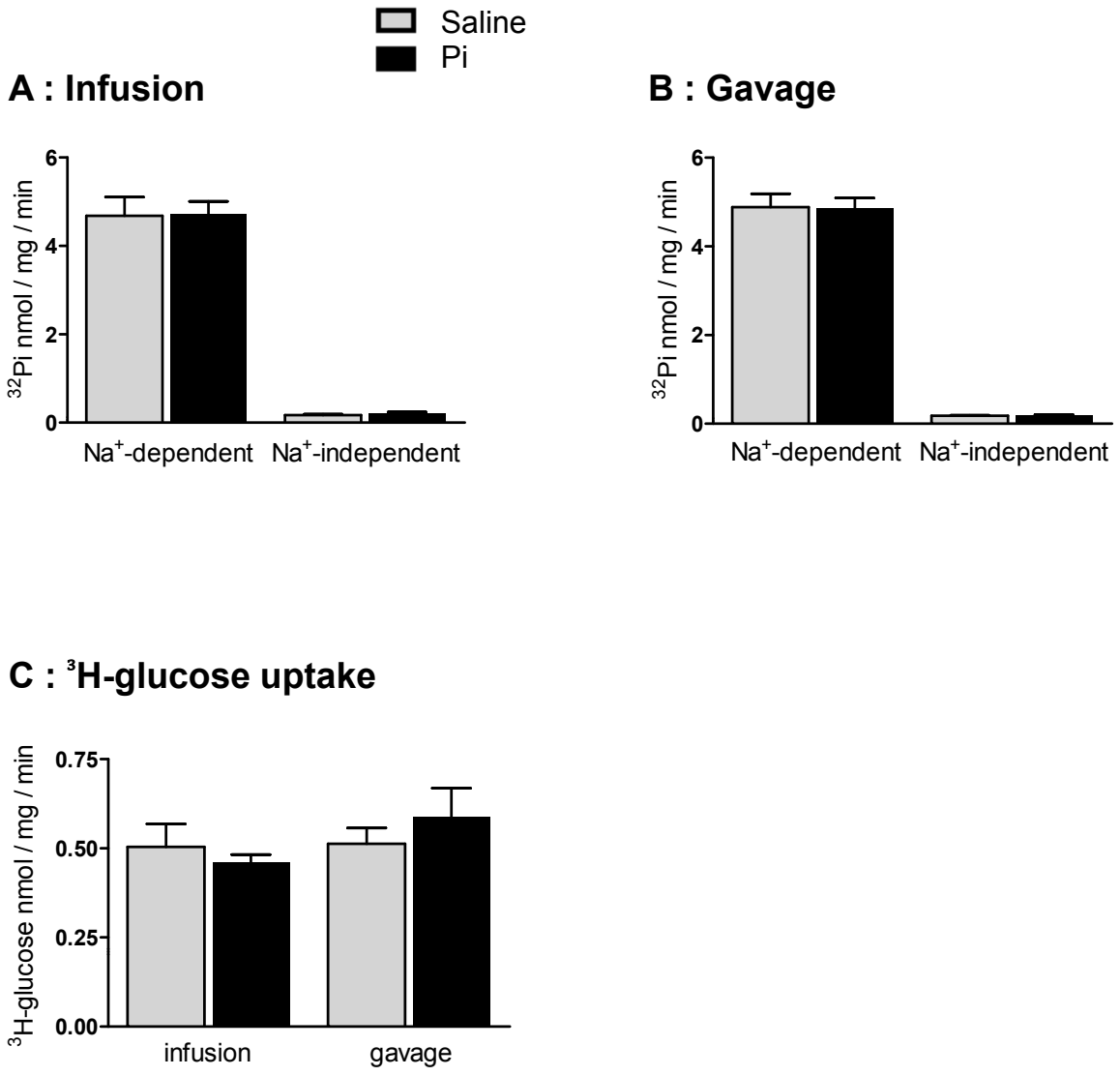
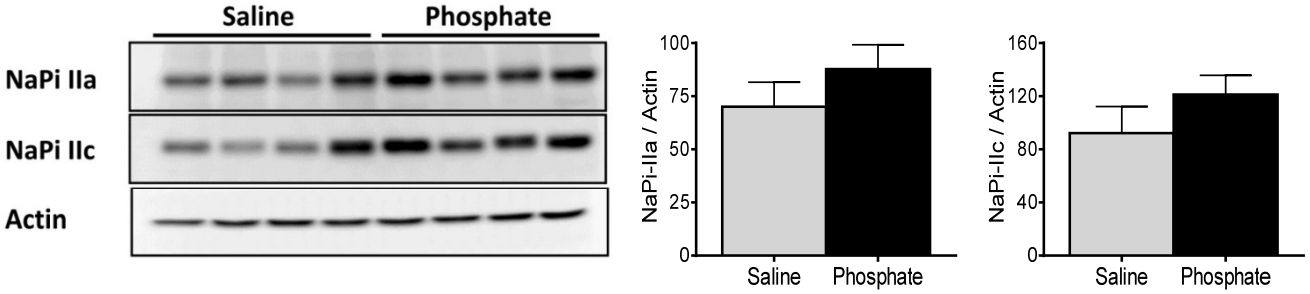
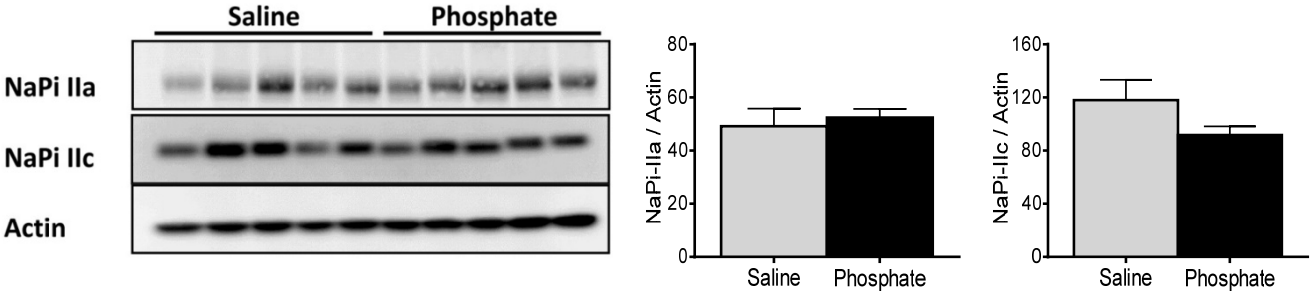

Saline
Pi
A : Infusion
B : Gavage
^{32}Pi nmol / mg / min
6
4
2
0
Na$^+$-dependent
Na$^+$-independent
C : ^{3}H-glucose uptake
^{3}H-glucose nmol / mg / min
0.75
0.50
0.25
0.00
infusion
gavage

Figure 10

A : Infusion



B : Gavage



Parathyroid hormone is critical for the acute adaption to oral or intravenous phosphate loading

Linto Thomas, Carla Bettoni, Thomas Knöpfel, Nati Hernando, Jürg Biber, and Carsten A. Wagner

Institute of Physiology, University of Zurich, Switzerland and National Centre for Competence in Research NCCR Kidney.CH, Switzerland

SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Effect of different doses of intravenous Phosphate loading on several parameters in intact rats

Rats were loaded with 50, 150, or 500 μmoles Pi intravenously (**A)** plasma Pi, (**B)** urinary Pi/creatinine, (**C**) plasma Ca^{2+}, **D**) urinary Ca^{2+}/creatinine, (**E**) intact PTH. Three profiles are shown: 50 μmoles Pi (black), 150 μmoles Pi (blue), 500 μmoles Pi (red). Data are presented as the mean ± SEM. **P*< 0.05 or ***P* <0.01 or *** *P*< 0.001 vs. *time 0*. Anova test, n = 5-9/group and time point.

Supplementary Figure 2. Effect of different concentrations of intravenous and intragastric administration of NaCl on several parameters in intact rats

Rats were loaded with 150 or 500 μmoles NaCl intravenously or orally. (**A)** plasma Pi, (**B)** urinary Pi, (**C**) plasma Ca^{2+} , **D**) urinary Ca^{2+}. Four profiles are shown: intravenous 150 μmoles NaCl (black), intravenous 500 μmoles NaCl (red), intragastric 150 μmoles NaCl (green), intrasgrastic 500 μmoles NaCl (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*. Anova test, n = 5-9/group and time point.

Supplementary Figure 3. Effect of intravenous and intragastric administration of Pi on fractional Pi excretion in intact and parathyroidectomized rats

Rats were loaded with 500 μmoles Pi or saline intragastrically or intravenously. (**A**) FE (%) for Pi in intact rats, (**B**) FE (%) for Pi in parathyroidectomized rats. Four profiles are shown: saline intravenous infusion (black), Pi intravenous infusion (red), saline intragastric gavage (green) and Pi intragastric gavage (blue). Data are presented as the mean ± SEM. **P*< 0.05 vs. *time 0*, Anova test, n = 4-9/group and time point.

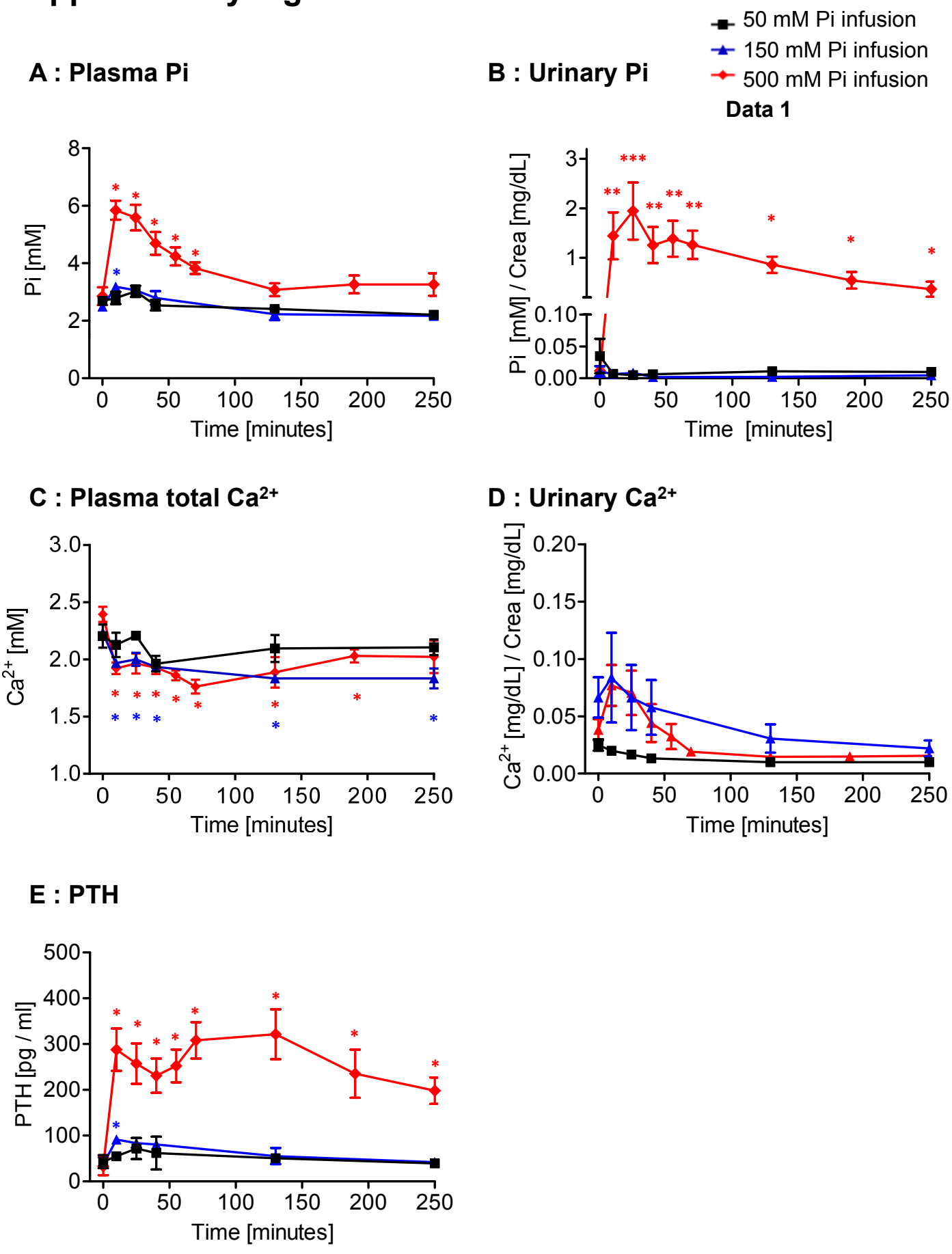
Supplementary Figure 4. Effect of intravenous and intragastric administration of phosphate on body phosphate distribution in intact rats

Rats were loaded with 500 μmoles Pi or saline intravenously or orally. **(A)** Concentration of Pi in femur, (**B**) concentration of Pi in skeletal muscle, (**C**) concentration of Pi in liver. Data are presented as the mean ± SEM. Anova test, n = 5-9/group and time point.

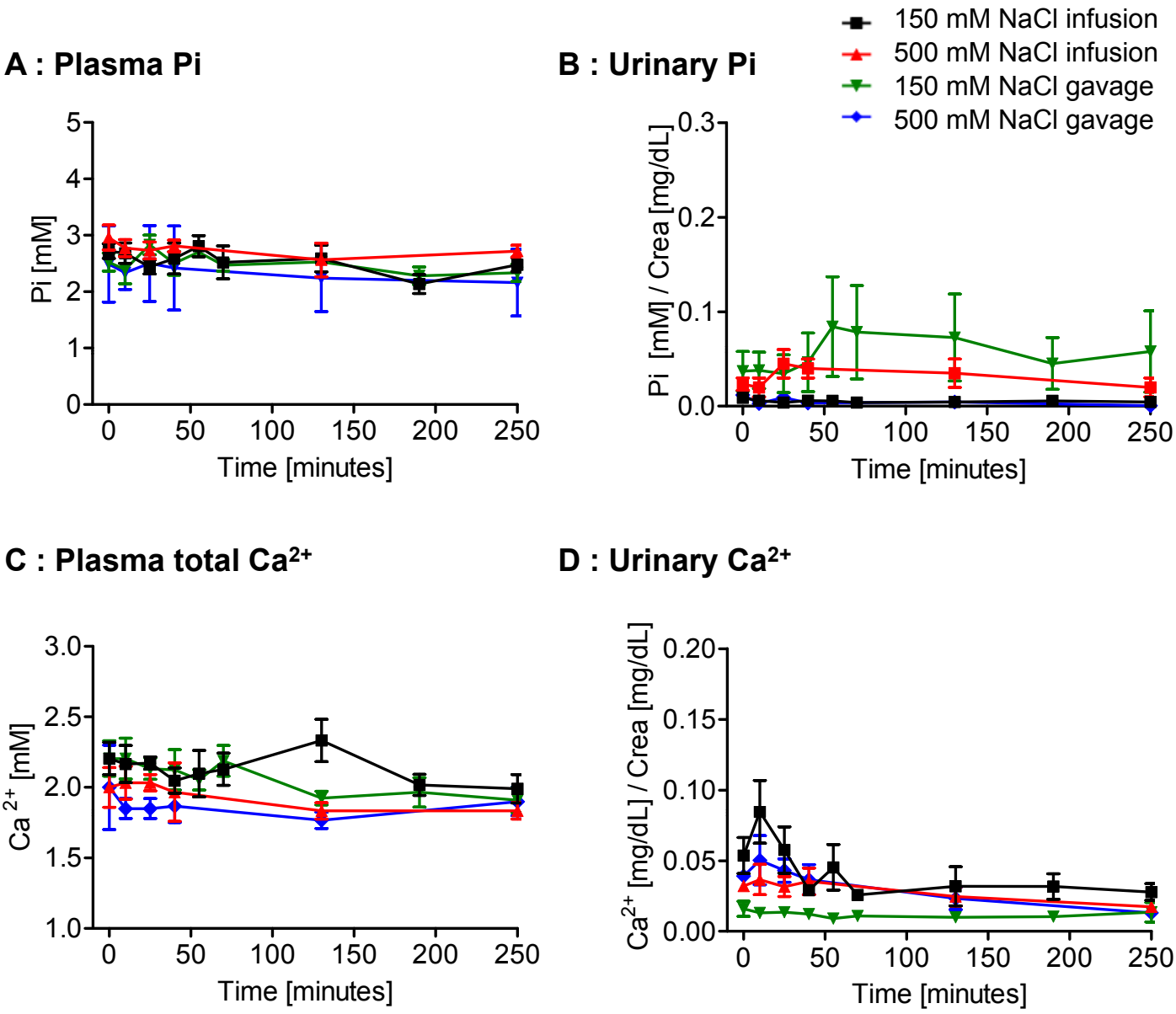
Supplementary Figure 5. Effect of intravenous and intragastric administration of phosphate on body phosphate distribution in parathyroidectomized rats

Parathyroidectomized rats were loaded with 500 μmoles Pi or saline intravenously or orally. (**A**) Concentration of Pi in femur, (**B**) concentration of Pi in skeletal muscle, (**C**) concentration of Pi in liver. Data are presented as the mean ± SEM, Anova test, n = 4-6/group and time point.

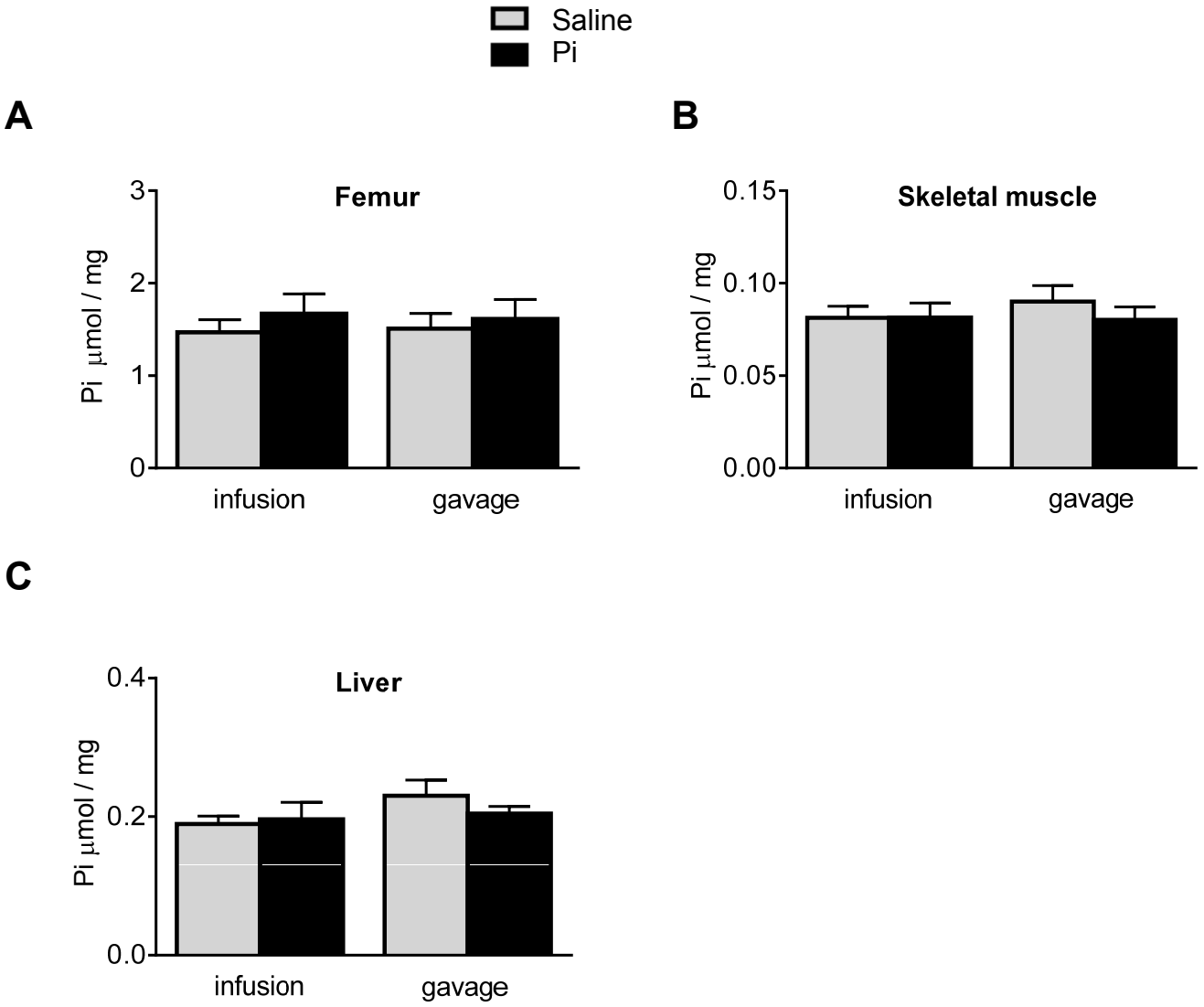
Supplementary Figure 1



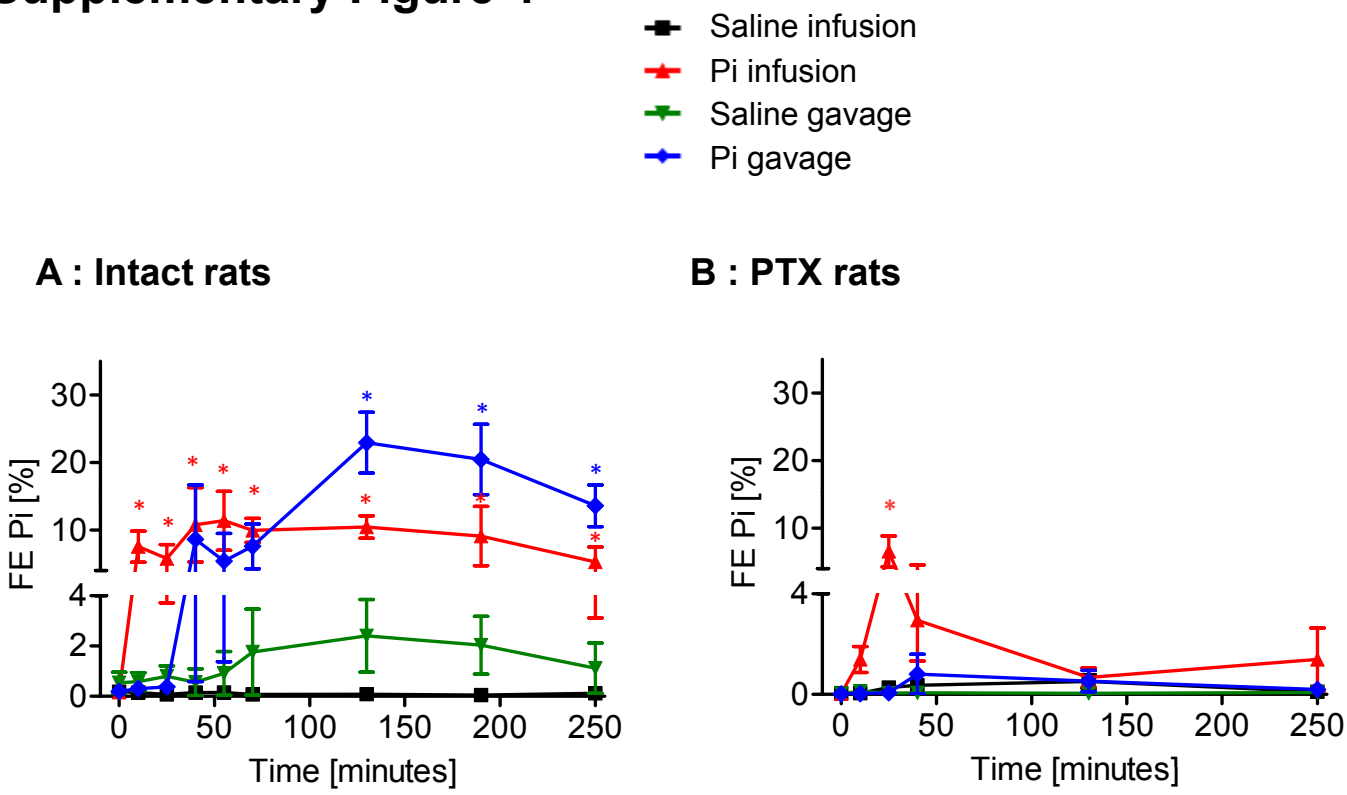
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5